



Antiviral Lectins of the Plant Family Amaryllidaceae

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Abstract

Plants have long served as a first line of defence against viral-borne diseases. Their chemical constituents have also afforded a sound basis for antiviral drug discovery. The plant family Amaryllidaceae is distinguished for its isoquinoline alkaloids, some of which have proved to be interesting antiviral drug leads. Its lectin (or agglutinin) principles have likewise attracted considerable attention as potential antiviral drugs. This review focuses on the antiviral activities that have been described for the lectins of the Amaryllidaceae. Of the thirty lectins known in the family, fourteen have been screened against nearly seventy pathogens belonging to thirteen viral families. Whilst good activities were reported in most cases, the lectins from *Galanthus nivalis*, *Narcissus pseudonarcissus* and *Hippeastrum* hybrid were identified with the best overall activities. They displayed potent inhibitory effects against the human immunodeficiency virus HIV-1(III_B) proliferation in CEM lymphoblastic cells (EC₅₀s 0.005, 0.009 and 0.004 μ M, respectively). Although significant effort was dedicated to the Retroviridae, noteworthy effects were also observed against members of other viral families (such as hepatitis C virus of the Flaviviridae). Furthermore, the lectins were shown to be highly selective antiviral agents, devoid of significant toxicities towards the nearly forty cells employed as hosts. Almost all of the details of their modes of operation have emerged from studies carried out on HIV. They were shown to inhibit viral attachment, fusion and adsorption to a variety of host cells. Modulation of viral entry was shown to occur via interference with the virus envelope glycoprotein. These observations fit into the key biological characteristic of lectins, that of sugar-binding proteins.

Keywords Antimicrobial · Antiviral · Drug discovery · Lectin · Mannose-specific/mannose-binding lectins · SARS-CoV-2 glycobiology

Introduction

Viruses have been prevalent amongst plants and animals since time immemorial (Strauss and Strauss 2007). On one hand associated with common ailments such as colds, influenza and chickenpox, on the other they are responsible for killer diseases such as Ebola, HIV/AIDS and rabies (Strauss and Strauss 2007). COVID-19 (coronavirus disease-19) has afforded a snapshot view of the deadly impact they can have on society, with over 6 million deaths reported by the third year of the outbreak (Koley and Dhole 2020; WHO 2022). There are close to 200 viral families, whose

members have been classified according to their nucleic acid content, strandedness, sense and method of replication (Strauss and Strauss 2007). The primary host defence against viruses is the immune response, which may involve innate and/or adaptive responses (Richman and Nathanson 2016). Engagement can also occur via cell-mediated responses with the involvement of T cells, interferon and macrophages to destroy infected host cells (Richman and Nathanson 2016). Vaccines and antiviral drugs have also played pivotal roles in reducing the burden of disease, as with polio, smallpox, mumps, herpes and HIV/AIDS (Richman and Nathanson 2016).

Plants have long been used as a first line of defence against viruses (Dhama et al. 2018). This together with their natural abundance and chemical diversity have afforded an ideal platform for plant-based antiviral drug discovery (Ali et al. 2021). Accordingly, antiviral activities have been described for most, if not all, classes of natural products (Ali et al. 2021). An often neglected area in the field is

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the proteinaceous constitution of plants, particularly their lectin principles (Komath et al. 2006). Also referred to as sugar-binding proteins, lectins are believed to be involved in seed survival and germination in plants (Komath et al. 2006). Two of the more conspicuous plant lectins include ricin from rice *Ricinus communis* L., Euphorbiaceae, and concanavalin A from the ‘jack bean’ *Canavalia ensiformis* (L.) DC., Fabaceae (Komath et al. 2006). Given their binding affinities, plant lectins are used in clinical settings for blood typing (Komath et al. 2006). There is also considerable interest in the exploitation of such properties for site-specific drug delivery (Raposo et al. 2021). A large number of lectins are to be found in humans, which perform roles in the agglutination of various cells, precipitation of glycoconjugates and polysaccharides, cell-to-cell interactions, signalling pathways and innate immune responses (Sharon and Lis 2004; Manikandan et al. 2020). Some of them act as pattern-recognition receptors, notably of glycoproteins on the cell surfaces of invading microorganisms as well as glycans and nucleic acids of malignant, apoptotic or dead host cells (Mason and Tarr 2015; Coelho et al. 2017).

The plant family Amaryllidaceae J.St.-Hil. is distinguished for its isoquinoline alkaloids, which exhibit interesting structural features and biological activities (Bastida et al. 2006). Whilst the majority of pharmacological research undertaken on these entities has been dedicated to their anticancer and motor neuron-related action (Bastida et al. 2006), there has nevertheless been sustained interest in their antiviral potential (Nair and van Staden 2023). Thirty-six species of the family were identified with functions in traditional medicine pertaining to the treatment of viral-borne diseases (Nair and van Staden 2023). Promising activities were also reported for several of its isoquinoline alkaloids against various viral pathogens including narciclasine, lycorine, haemanthamine and pretazettine (Nair and van Staden 2023). The inhibition of viral DNA, RNA and protein syntheses was among the pathways by which they manifested their action (Nair and van Staden 2023). Given these interesting observations, this account focuses on the antiviral activities that have been described for the lectin constituents of the Amaryllidaceae. Significant effort is also made to uncover the mechanisms which underpin these effects.

Search Strategy

This survey covers material published on the antiviral activities of Amaryllidaceae lectins, from the first report in 1987 up to and including 2022. Five search engines were utilized during the literature search including Google Scholar, PubMed, SciFinder, Scopus and Web of Science. The key search terms engaged were ‘Amaryllidaceae lectin’ and ‘antiviral Amaryllidaceae lectin’. Once the chief antiviral

Amaryllidaceae lectins were identified as ‘*Galanthus nivalis* agglutinin’ (GNA), ‘*Narcissus pseudonarcissus* agglutinin’ (NPA) and ‘*Hippeastrum hybrid* agglutinin’ (HHA), further searches were made using them as search items. These together returned over three hundred hits, of which around eighty were adjudged to be of direct relevance to the theme of the text. Information was first grouped according to lectin type and then by activities based on the different viral families, and is presented as such in the text.

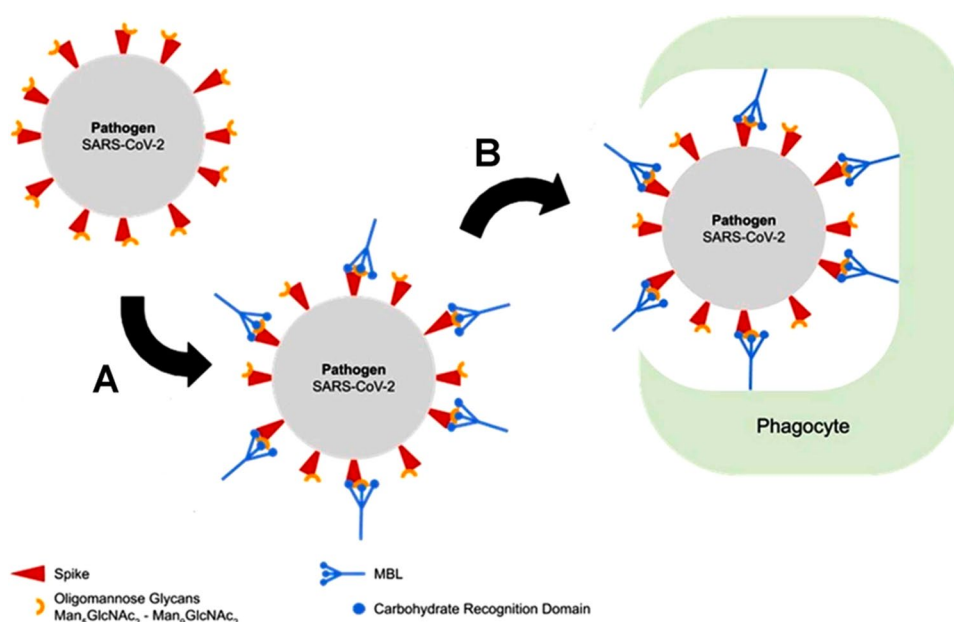
Discussion

Mechanisms of Antiviral Lectins

The innate immune system is the first line of defence against infection (Strauss and Strauss 2007). A part of this system includes mannose-binding lectins (MBLs), which are a superfamily of collagen-containing C-type lectins (Lau 2020). Mannose-binding lectins are proteins produced by the liver and secreted into the serum where they can trigger an immune response (Lau 2020). Mannose-binding lectins have carbohydrate recognition domains (CRDs) which facilitate their binding to infectious agents such as bacteria and viruses, thereby activating the complement cascade of the immune system (Lau 2020). Mannose-binding lectins enhance phagocytosis by stimulating binding and complement activation as well as acting as opsonins via the lectin pathway (Fig. 1) (Lau 2020). Complement activation also serves as a bridge to the adaptive immune system for the production of antigen-specific antibodies, through which acquired immunity can be attained (Lau 2020). The molecular interaction between a lectin and its carbohydrate substrate is highly specific, allowing for the recognition of both monomeric sugars as well as oligosaccharides that constitute branched high-mannose or complex glycans (Mitchell et al. 2017). Given that oligosaccharide-based post-translational modifications of proteins are ubiquitous through all forms of life, lectins have evolved to perform manifold roles in organismal biology such as self-recognition, protein folding, and cell movement and adherence (Mitchell et al. 2017). Glycosylated envelope proteins (GEPs) are the key viral proteins involved in recognition and entry into the host (Ahmed et al. 2022). They exhibit strong affinities for the cell-surface proteins of host cells (Ahmed et al. 2022). Glycosylated envelope proteins reflect significant levels of high-mannose-type glycans (HMGs), which buffers them from antibody neutralization and enables them to interact with cell entry receptors (Nabi-Afjadi et al. 2022). By reacting with high-mannose glycans, antiviral lectins can also trigger the glycosylation of viral GEPs (Ahmed et al. 2022). Such structures aid in viral evasion of the immune system and entry into host cells, which is also mediated by recognition

Fig. 1 The lectin pathway.

Mannose-binding lectins, key molecules in the innate immune response, contribute to the host defence against coronaviruses such as SARS-CoV. **A** Opsonization, from the Greek *opsōnein* meaning to prepare for eating. MBLs use their carbohydrate recognition domains to bind to the exposed mannose glycans on SARS-CoV-2. **B** Phagocytosis, from ancient Greek *phagein* meaning to eat. Phagocytes recognize MBL-bound SARS-CoV-2, ingest the pathogen and digest it. Reproduced from Lau (2020)



of the CD4+ trigger (Ahmed et al. 2022). Antiviral lectins also inhibit the conformational reorganization of the GEP complex, which results in the suppression of viral entry into host cells (Ahmed et al. 2022).

Bioavailability

A significant hurdle to overcome in the clinical development of antiviral lectins is the assessment of their bioavailabilities (Mitchell et al. 2017). Considering they are proteins, it stands to reason that lectins could not follow an oral route of administration as they would not survive the enzymes of the digestive system (Mitchell et al. 2017). Surprisingly few studies have attempted to assess the systemic bioavailability of lectins (Mitchell et al. 2017). For example, the cyanobacterial lectin cyanovirin-N (CV-N) was biologically available following subcutaneous injection into mice, with doses up to 5.6 mg/kg/day shown to be well tolerated (Mitchell et al. 2017). Understandably less information is available on such effects for the Amaryllidaceae lectins. However, one such study involved a screen of NMRI mice for susceptibilities to GNA and HHA (Balzarini et al. 2004a). When injected intravenously into the tail with GNA (100 mg/kg) or HHA (50 mg/kg) as a bolus injection, none of the animals displayed signs of acute toxicity or visible toxic side effects for at least 5 days post-treatment (Balzarini et al. 2004a).

Toxicity and Immunogenicity

A serious concern in the development of any clinical agent is the possibility that toxicities may arise that are unrelated to the target of the active agent. Although the

oligosaccharides on viral GEPs are the targets of antiviral lectins, they are invariably synthesized by and attached to host cell enzymes (Mitchell et al. 2017). This suggests that they can also be present in human cells. Potential deleterious effects from the systemic use of lectins could be borne out of their renowned abilities to agglutinate cells (Mitchell et al. 2017). In regard to such effects for Amaryllidaceae lectins, both GNA and NPA were capable of agglutinating trypsin-exposed rabbit erythrocytes but not human red blood cells (RBCs), a reaction that was reversible by mannose (Van Damme et al. 1987, 1988). Further toxicities could potentially be manifested via acute immunological responses to the administration of a foreign protein (anaphylaxis) (Mitchell et al. 2017). For example, concanavalin A was shown to be responsible for acute liver damage in mice due to it binding to sinusoidal endothelial cells, which caused them to be attacked by CD4+ T cells (Mitchell et al. 2017). Nonetheless, there are examples for the successful use of heterologous proteins in the management of human conditions, such as the cone snail peptide ziconotide for chronic pain, the Gila monster saliva peptide exenatide for type II diabetes and the microbial product botulinum toxin in cosmetic therapy (Mitchell et al. 2017). Heterologous proteins also have the potential to stimulate the immune system, such as the activation of T cells or PBMCs (Mitchell et al. 2017). This can, in HIV for example, stimulate CD4+ HIV target cells close to sites of administration, which can have the reverse effect of actually raising the probability of infection (Mitchell et al. 2017; Naik and Kumar 2022). This should be of some concern for Amaryllidaceae lectins since their greatest potential appears to reside in the area of HIV therapeutics.

Administration

Oral means of administration of lectins have been precluded by virtue of their proteinaceous characteristics (Mitchell et al. 2017). Efficacies of antiviral proteins against various respiratory infections have nevertheless been demonstrated via deliveries made topically, systemically (by subcutaneous or intraperitoneal injection) and by intranasal presentation (Mitchell et al. 2017). Vaginal administration has also been shown to be a viable route; for example, in the delivery of genetically engineered lactobacilli secreting CV-N (Mitchell et al. 2017). It remains to be clarified what mode of administration would suit the delivery of Amaryllidaceae lectins.

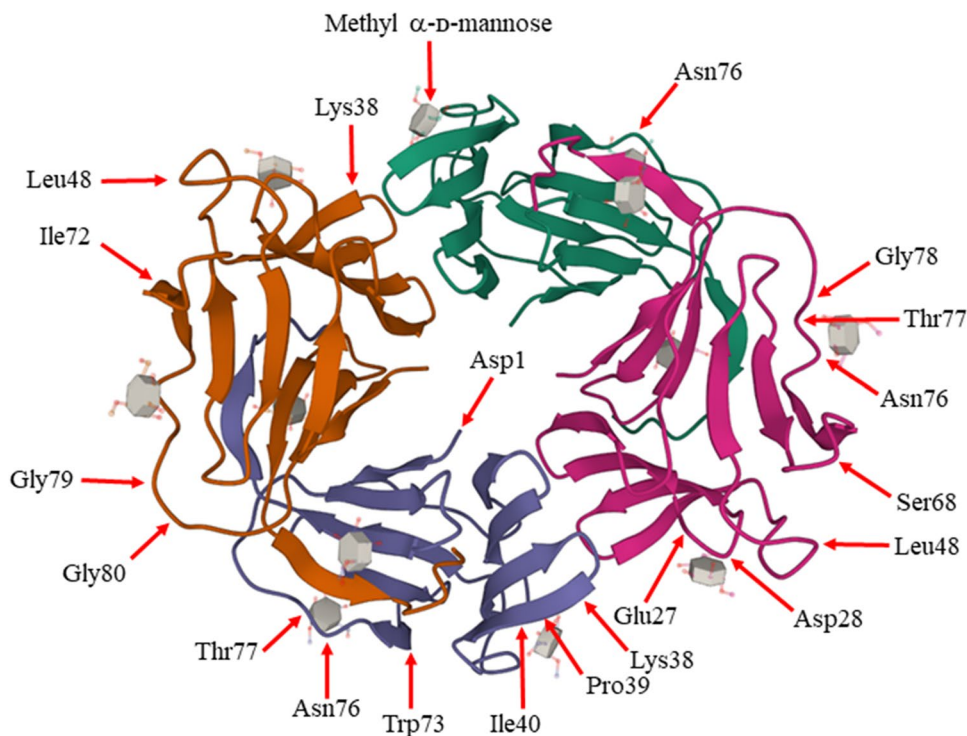
Galanthus nivalis Agglutinin

Galanthus nivalis agglutinin (GNA) was the first lectin to be described from the Amaryllidaceae (Van Damme et al. 1987). It was identified as a tetrameric protein composed of four identical subunits (Fig. 2), each with a relative molecular weight M_r of 13 kDa (Van Damme et al. 1987; Hester et al. 1996). Labelling experiments indicated that GNA was synthesized in the endoplasmic reticulum as a precursor with an M_r of 15 kDa and converted post-translationally into the authentic lectin polypeptide (M_r 13 kDa) (Van Damme and Peumans 1988). The molecular structure of GNA is pH-dependent as it was shown to exist as a dimer at pHs of 5 and 9, respectively (Van Damme et al. 1988). Amino acid analysis indicated significant representation of asparagine/

aspartic acid, glycine, serine and leucine residues, without the presence of methionine (Van Damme et al. 1987). GNA exhibited exclusive mannose-binding specificity (Van Damme et al. 1987). It readily agglutinated trypsin-treated rabbit erythrocytes, but not human red blood cells (RBCs), which could be reversed by mannose (Van Damme et al. 1987). Agglutination was maintained after reduction with β -mercaptoethanol, suggesting that the binding of GNA subunits did not involve disulphide bridges (Van Damme et al. 1987). Furthermore, it was shown that GNA precipitated branched yeast mannans, without affecting any glucans, and that D-mannose (but not D-glucose) was an inhibitor of the GNA-mannan interaction (Shibuya et al. 1988). Inhibition experiments with various sugars revealed that GNA required the presence of equatorial hydroxyl groups at the C-3 and C-4 positions and an axial group at the C-2 position of the D-pyranose ring (Shibuya et al. 1988). A non-reducing terminal D-mannose residue was necessary for the interaction of oligosaccharides, of which those with terminal man(α -1,3)man units were the most inhibitory (10–30 times more so than D-mannose) (Shibuya et al. 1988).

Following this, GNA was examined for antiviral effects against the retrovirus HIV, where it was active against both HIV-1(III_B) and HIV-2(LAV-2) (EC₅₀s 0.4 and 0.3 μ g/ml, respectively) cultured in human MT-4 cells (Table S1), proving to be as effective as dextran sulphate 10,000 (DS-10000) (EC₅₀ 0.4 μ g/ml) (Balzarini et al. 1991). A further feature of its reactivity towards HIV was the fact that GNA suppressed syncytium formation between HIV-1- or HIV-2-infected

Fig. 2 Crystal structure of GNA (resolved at 2.3 Å) complexed with methyl α -D-mannose showing the four independent subunits of the tetramer as well as selected amino acid residues. Structure available free of charge at the Protein Data Bank via the PDB <http://doi.org/10.2210/pdb1MSA/pdb>; see also Hester et al. (1996)



HuT-78 lymphocytes and uninfected Molt-4 lymphoblasts (EC_{50} s 2.7 and 3.0 $\mu\text{g/ml}$, respectively), much more so than DS-5000 was able to (EC_{50} 25 $\mu\text{g/ml}$) (Balzarini et al. 1991). Whilst GNA did not inhibit OKT4A from binding to MT-4 cells, it did inhibit anti-gp120 binding to HuT-78/HIV-1 cells (47% inhibition after 15 min) (Balzarini et al. 1991). The monoclonal antibody OKT4A is used as a phenotypic marker for CD4 expression (Tubo and Jenkins 2014). Found on the surface of immune cells such as T-helper cells, monocytes, macrophages and dendritic cells, CD4 functions as a co-receptor for the T cell receptor (Tubo and Jenkins 2014). The glycoprotein gp120 is found on the surface of the HIV envelope (Env) and undergoes distinct conformational changes upon binding to CD4, which lead to the fusion of the viral membrane with the host cell membrane (Tubo and Jenkins 2014). MT-4 cells that adsorbed HIV-1 particles were distinguishable from those that did not via immunofluorescence with polyclonal anti-HIV antibodies (Balzarini et al. 1991). Whilst DS-5000 (at 25 $\mu\text{g/ml}$) completely blocked HIV-1 adsorption, GNA only became active at a concentration of 100 $\mu\text{g/ml}$ (or higher) suggesting that it did not markedly inhibit HIV-1 binding to CD4+ cells (Balzarini et al. 1991). Overall, the data suggested that GNA interfered with the HIV replicative cycle via an event that was subsequent to the attachment (*i.e.* the fusion process) of the virions to the cells (Balzarini et al. 1991).

MT-4 cells were also exploited as hosts to probe the effects of GNA on two clinical isolates of HIV-1: the lymphadenopathy-associated virus (LAV) and nucleoside diphosphate kinase-associated (NDK) isolates (Hammar et al. 1994). Pretreatment of the virus with GNA (5 $\mu\text{g/ml}$ –5 ng/ml) resulted in the neutralization of both isolates to the same extent (Hammar et al. 1994). Pretreatment of MT-4 cells also offered significant protection from infection, but in which case the NDK isolate was about 100-fold more sensitive to treatment than the LAV isolate (Hammar et al. 1994). The fact that α -mannosidase-treated cells were not protected confirmed the requirement of terminal mannose residues for GNA to exert its effects, also suggesting that such residues were not receptors for the virus (Hammar et al. 1994). From the overall effects of GNA in MT-4 cells it was suggested that the lectin may prevent infection by binding to glycoproteins on the surface of infected cells (Hammar et al. 1994). However, the observations made with the pretreatment of non-infected cells suggested that there could be other factors at play, such as the binding of structures in the vicinity of essential receptors which would affect the interaction of the virus with the receptor (Hammar et al. 1994).

Further examination of the effects of GNA on HIV-1(III_B) involved the use of C8166 T cell leukaemia cells as host (Animashaun et al. 1993). Syncytium formation relative to 3'-azido-2',3'-dideoxythymidine (AZT) was inhibited by GNA at both 10 and 100 $\mu\text{g/ml}$, with the lectin shown to

be ineffective at the lowest tested dosage (1 $\mu\text{g/ml}$) (Animashaun et al. 1993). GNA significantly depleted viral multiplication, with new colonies (50%) only observable at the lowest tested concentration (Animashaun et al. 1993). Uninfected C8166 cells were fully viable at all three tested dosages, which was reduced to 63% in infected cells only at the lowest dosage, suggesting that GNA posed no cytotoxic threat to the host (Animashaun et al. 1993). GNA markedly inhibited p24 production in HIV-infected cells with 107% of the antigen detected at the lowest tested dosage (0.016 $\mu\text{g/ml}$), which fell in a dose-dependent manner to 12% at 2 $\mu\text{g/ml}$ and 0% with the two remaining concentrations (50 and 100 $\mu\text{g/ml}$, respectively) (Animashaun et al. 1993). The HIV antigen p24 is a protein that makes up most of the HIV capsid (Animashaun et al. 1993). The onset of AIDS correlates with a reduction in the number of CD4+ T cells and increased levels of virus and p24 in the blood, making it a useful handle for diagnostic purposes (Animashaun et al. 1993). Analysis of bound glycoproteins from GNA-sepharose beads that were mixed with either infected or uninfected C8166 cell lysates indicated (via Western blotting and gp120-specific antibodies) the binding of gp120 (Animashaun et al. 1993). GNA has also been used in lectin affinity chromatography to purify the envelope glycoproteins of HIV-1, HIV-2 and SIV (simian immunodeficiency virus), which were subsequently shown to exhibit CD4-binding and antigenic reactivities (Gilljam 1993). Following immunization with these preparations, strong immune responses to envelope proteins and peptides were observed in mice and primate subjects (Gilljam 1993).

Galanthus nivalis agglutinin (in CEM lymphoblastic cells) was also noted for its response to several drug-resistant HIV strains including non-nucleoside reverse transcriptase inhibitor NNRTI-resistant HIV-1 (EC_{50} 0.85 $\mu\text{g/ml}$), lamivudine-resistant HIV-1 (EC_{50} 0.40 $\mu\text{g/ml}$), zidovudine-resistant HIV-1 (EC_{50} 1.0 $\mu\text{g/ml}$) and protease inhibitor-resistant HIV-1 (EC_{50} 0.33 $\mu\text{g/ml}$) (Balzarini et al. 2004a). The mean EC_{50} established for tenofovir over these four cases was 8.45 $\mu\text{g/ml}$ (Balzarini et al. 2004a). Further studies of the virus in CEM cells involved the HIV-1(III_B) strain against which GNA displayed a potent effect (EC_{50} 0.013 μM), over 30 times more so than DS-5000 (EC_{50} 0.45 μM) (Bertaux et al. 2007). An interesting observation made here was that the effect could be attenuated by the inclusion of the mannose polysaccharide mannan (2.5 mg/ml) to the culture medium, so that the half effective concentration dropped to as low as 0.54 μM (Bertaux et al. 2007). This reinforced the notion for the affinity of CBAs (carbohydrate-binding agents) with viral envelope-associated glycans (Bertaux et al. 2007). The EC_{50} drop for DS-5000 in the same instance was only by 0.15 μM (Bertaux et al. 2007). HIV-2 strain ROD was also strongly inhibited by GNA

(EC₅₀ 0.011 µM) relative to DS-5000 (EC₅₀ 0.16 µM), with negligible effects in each case manifested on CEM cells (CC₅₀s > 2 and > 100 µM, respectively) (Bertaux et al. 2007). GNA efficiently prevented infection of MT-4 cells by HIV-1 strains that were resistant to the virus adsorption inhibitor DS-5000 and the virus entry inhibitors AMD-3100 and SDF-1, with EC₅₀s of 0.13, 0.59 and 0.60 µg/ml, respectively (Balzarini et al. 2004a). The corresponding wild-type HIV-1 strain NL4.3 also succumbed to exposure to GNA (EC₅₀ 0.57 µg/ml), but at a markedly higher level than by DS-5000 (EC₅₀ 0.14 µg/ml) or AMD-3100 (EC₅₀ 0.005 µg/ml) (Balzarini et al. 2004a). Its inhibition of syncytium formation between HIV-1- or HIV-2-infected HuT-78 and uninfected lymphoblastic leukaemia Sup-T1 cells was much more pronounced for the former than the latter (EC₅₀s 1.25 and 80 µg/ml, respectively) (Balzarini et al. 2004a). Standard DS-5000 also exhibited a similar discriminatory response in this coupled cell system (EC₅₀s 2.7 and 10 µg/ml, respectively) (Balzarini et al. 2004a). GNA did not interfere with CD4, CXCR4 (C-X-C chemokine receptor type 4), CCR5 (C-C chemokine receptor type 5) or DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin) expression neither did it specifically bind to the membranes of uninfected cells, suggesting that it is likely to interrupt virus entry by interfering with the virus envelope glycoprotein (Balzarini et al. 2004a). The infection of CD4 + T lymphocytic C8166 cultures by HIV-1(NL4.3) was strongly inhibited by GNA as shown by flow cytometric analysis (EC₅₀ 0.041 µg/ml) and microscopic scoring (EC₅₀ 0.045 µg/ml) (Petrova et al. 2013). Pradimicin-A by contrast reflected EC₅₀ values of 4.4 and 3.9 µg/ml via these two methods of analysis, respectively (Petrova et al. 2013). GNA also prevented syncytium formation between infected HuT-78 lymphocytes and uninfected Sup-T1 co-cultures (EC₅₀ 0.47 µg/ml) (Petrova et al. 2013). Furthermore, the capture of viral particles by DC-SIGN + Raji cells via gp120 was dose-dependently suppressed by GNA, with 50% inhibition observed at a dosage of 5.13 µg/ml (*i.e.* 125 times the EC₅₀) (Petrova et al. 2013). The transmission of Raji/DC-SIGN-captured viral particles to uninfected C8166 cells was also dose-dependently inhibited by GNA (50% inhibition at its EC₅₀ value) (Petrova et al. 2013). Further studies indicated that GNA could be used synergistically with the commercial drug tenofovir against HIV-1(NL4.3) (Ferir et al. 2011). Individual assessments showed the EC₅₀ values against viral replication in MT-4 cells to be 0.014 and 6.7 µM, respectively (Ferir et al. 2011). The EC₅₀ values dropped to 0.0046 and 1.5 µM, respectively, in cells exposed to equimolar quantities of GNA and tenofovir (Ferir et al. 2011). Similar effects were observed for HIV-1(BaL) infection of peripheral blood mononuclear cells (PBMCs), where EC₅₀ values for GNA

and tenofovir were 0.15 and 1.2 µM, respectively (Ferir et al. 2011). In combination at the same concentrations, these were determined to be 0.04 and 1.0 µM, respectively (Ferir et al. 2011).

Exposure of HIV-1 to DC-SIGN-expressing B-lymphoblastic Raji cells (Raji/DC-SIGN) resulted in the capture of HIV-1 particles as measured by p24 antigen expression (Balzarini et al. 2007). The process was inhibited by GNA (IC₅₀ 18 µg/ml), much more so than by AMD-3100 or DS-5000 (IC₅₀s > 50 and > 250 µg/ml, respectively) (Balzarini et al. 2007). By contrast, wild-type Raji/0 cells did not suffer the same fate when exposed to HIV-1 (Balzarini et al. 2007). GNA also inhibited syncytium formation in HIV-1(III_B)-infected C8166 CD4 + T lymphocytes (EC₅₀ 20 µg/ml) as well as between Raji/DC-SIGN and uninfected C8166 cells (EC₅₀ > 20 µg/ml), but to a far lesser extent than by either AMD-3100 (mean EC₅₀ 0.44 µg/ml) or DS-500 (mean EC₅₀ 2.80 µg/ml) (Balzarini et al. 2007). The C-type lectin receptor DC-SIGN is present on the surface of both macrophages and dendritic cells, where in the former it binds to high-mannose type *N*-glycans found commonly in viruses, bacteria and fungi (Balzarini et al. 2007). DC-SIGN-or L-SIGN-expressing 293 T-REx cells were readily infected by HIV-1(NL4.3-EGFP) (Auwerx et al. 2009). However, viral capture by these human embryonic kidney cells was effectively negated by GNA (EC₅₀s 0.098 and 0.070 µM, respectively) (Auwerx et al. 2009). The responses were seen to be much more pronounced compared to pradimicin-A (EC₅₀s 35 and 11 µM, respectively) or DS-5000 (EC₅₀s > 250 and 37 µM, respectively) (Auwerx et al. 2009). GNA also dose-dependently inhibited the formation of syncytia in co-cultures of HIV-1-captured 293 T-REx cells (expressing DC- or L-SIGN) and uninfected C8166 CD4 + T lymphocytes (Auwerx et al. 2009). L-SIGN, like DC-SIGN, is a cell adhesion and receptor protein that mediates in cellular interactions and the recognition of a wide variety of pathogens (Auwerx et al. 2009).

When exposed to increasing concentrations of GNA, a variety of HIV-1(III_B) strains were identified after 20 to 40 subcultivations which exhibited diminishing sensitivities to the lectin (Balzarini et al. 2004b). EC₅₀ values ascertained for GNA against the mutant strains GNA-1.0, GNA-1.1a and GNA-1.2 (produced at lectin concentrations of 0.5, 10 and 100 µg/ml, respectively) were 1, 12.2 and 48.8 µg/ml in CEM cells (Balzarini et al. 2004b). GNA exhibited an EC₅₀ of 0.4 µg/ml against wild-type HIV-1(III_B), whilst the mean value for tenofovir over the four strains was 4.8 µg/ml (Balzarini et al. 2004b). The mutant virus isolates reflected changes to amino acids in the envelope glycoprotein gp120, but not in gp41 (Balzarini et al. 2004b). The majority of these changes occurred at *N*-glycosylation sites and at serine or threonine sites that were part of a glycosylation motif (Balzarini et al. 2004b). It was shown further that whilst

GNA was active against wild-type HIV-1(BRU) (EC_{50} 0.15 $\mu\text{g/ml}$), it was close to 30 times less effective against its clone that was mutated at several *N*-glycosylation sites in the LA loop of gp120 (Balzarini et al. 2004b). A further HIV-1(III_B) mutant identified in CEM cell cultures exposed to increasing GNA concentrations (0.25–500 $\mu\text{g/ml}$) was strain GNA-500CS (Balzarini et al. 2005a). The mutations involved asparagine or threonine/serine residues at *N*-glycosylation sites of gp120, but not at those associated with gp41 (Balzarini et al. 2005a). The glycosylation sites were also clustered in regions of gp120 that showed no interactions with either CD4, CCR5 or CXCR4 (Balzarini et al. 2005a). GNA-500CS was shown to be highly resistant to GNA (EC_{50} 157 $\mu\text{g/ml}$) compared to wild-type HIV-1(III_B) (EC_{50} 0.27 $\mu\text{g/ml}$) (Balzarini et al. 2005a). It was also resistant to the monoclonal antibody 2G12 (which binds to an HIV-1 oligomannose epitope of gp120), modestly resistant to mannose-specific cyanovirin-N (from blue-green algae), but fully susceptible to HIV entry inhibitors (DS-5000, AMD-3100, T-20) as well as HIV reverse transcriptase inhibitors (UC-781, tenofovir) (Balzarini et al. 2005a). A constantly evolving glycan shield within the viral envelope is a strategy HIV uses to evade immune surveillance (Balzarini et al. 2005a). Plant lectins such as GNA may thus force the virus to convert to a gp120 phenotype that cannot properly escape the immune response, which in turn may result in a more efficient elimination of HIV from the body (Balzarini et al. 2005a).

Further studies on the HIV-1(III_B) mutant GNA-500CS showed that its resistance to GNA could be overcome synergistically with the inclusion of the $\alpha(1,2)$ -mannosidase I inhibitor 1-deoxymannojirimycin (DMJ) (Balzarini 2007). Thus, whilst GNA at doses of 4 and 20 $\mu\text{g/ml}$ showed poor efficacy against the mutant in CEM cultures, it became 100% protective in the presence of 50 μM DMJ and 40–100% protective with the addition of 20 μM DMJ (Balzarini 2007). Furthermore, DMJ alone was ineffective against both wild-type HIV-1(III_B) and mutant GNA-500CS (EC_{50} s > 500 and 90 $\mu\text{g/ml}$, respectively) (Balzarini 2007). The corresponding EC_{50} data for GNA alone were 0.45 and 103 $\mu\text{g/ml}$, respectively (Balzarini 2007). From this it was suggested that DMJ may be converting some of the remaining glycans of the mutant gp120 into high-mannose-type glycan structures, making them more vulnerable to the CBAs (Balzarini 2007).

The 8.5-kDa monomeric agglutinin (UDA) from the ‘common nettle’ *Urtica dioica* L., Urticaceae, displayed *N*-acetylglucosamine-binding specificity (Balzarini et al. 2005b). Subculturing of HIV-1(III_B)-infected CEM cells with varying concentrations of UDA (starting from 5 $\mu\text{g/ml}$) led to the production of seven mutant strains which, as seen for GNA above, reflected amino acid changes that were linked to gp120 (Balzarini et al. 2005b). Relative to its effect on wild-type HIV-1(III_B) (EC_{50} 0.018 μM), GNA

was close to 30 times less active against the seven mutant strains (mean EC_{50} 0.50 μM) (Balzarini et al. 2005b). Similarly, UDA was less active on the mutant strains (mean EC_{50} 1.28 μM) than it was against HIV-1(III_B) (EC_{50} 0.14 μM) (Balzarini et al. 2005b).

Mutated strains of HIV-1(III_B) were also produced in response to cyanovirin-N (CV-N) administered at increasing concentrations in CEM cells (Balzarini et al. 2006). These were shown to involve a total of eight amino acid mutations exclusively at *N*-glycosylation sites of gp120 on the envelope surface, six of which caused the deletion of high-mannose type *N*-glycans (Balzarini et al. 2006). The level of phenotypic resistance of the mutated virus strains to CV-N in general could be correlated with the number of glycan deletions in gp120 (Balzarini et al. 2006). For example, strains CV-N2a,113–114 and CV-N2b,113–115 with 6 mutations each were less sensitive (mean EC_{50} 0.040 μM) to CV-N than strain CV-N2,108–4 (EC_{50} 0.016 μM) which contained three mutations (Balzarini et al. 2006). By comparison, wild-type HIV-1(III_B) easily succumbed from exposure to CV-N (EC_{50} 0.002 μM) (Balzarini et al. 2006). The results for GNA against the mutated strains were much closer to each other (mean EC_{50} 0.096 μM , EC_{50} 0.14 μM) but lower than those seen with CV-N, which suggested the prevalence of a higher genetic barrier for CV-N, with the wild-type strain again seen to be most susceptible (EC_{50} 0.005 μM) (Balzarini et al. 2006).

The emergence of escape mutants that are no longer susceptible to drugs poses a considerable challenge to the treatment of HIV/AIDS (Martinez-Cajas and Wainberg 2008). Culturing of HIV-1(NL4.3) in MT-4 cells with increasing concentrations of cyclotriazadisulfonamide (CADA) produced a mutant that showed diminished susceptibility to the compound (Vermeire et al. 2009). Cyclotriazadisulfonamide is an HIV entry inhibitor that causes a reduction in surface CD4 in several types of human CD4+ cells (such as T lymphocytes, monocytes and dendritic cells), thus producing broad-spectrum antiviral potency (Vermeire et al. 2009). The mutant HIV-1 NL4.3 (CADARes40) reflected mutations in the C4 and V5 regions of gp120, which likely stabilized the CD4-binding conformation (Vermeire et al. 2009). Isolated after 40 passages through cell culture, CADARes40 was insensitive to the CD4 receptor-downmodulating effect of CADA (EC_{50} > 50 $\mu\text{g/ml}$), whilst the wild-type strain (after 40 passages through culture medium) remained sensitive (EC_{50} 1.2 $\mu\text{g/ml}$) (Vermeire et al. 2009). GNA by contrast inhibited multiplication of both the mutant (EC_{50} 0.10 $\mu\text{g/ml}$) and wild-type (EC_{50} 0.14 $\mu\text{g/ml}$) strains (Vermeire et al. 2009). These measurements were made relative to enfuvirtide (EC_{50} s 0.062 and 0.056 $\mu\text{g/ml}$, respectively) and indinavir (EC_{50} s 0.013 and 0.024 $\mu\text{g/ml}$, respectively) (Vermeire et al. 2009).

Amongst the ways HIV-1 can develop resistance to CBAs, the deletion of high-mannose and complex-type glycan residues in the C2–C4 regions of gp120 is most plausible (Wu and Bao 2013). However, the frequency of natural resistance to CBAs in transmitted founder (TF) viral strains is still unclear (Hu et al. 2015). TF viruses, which are identifiable early after HIV-1 transmission, are thought to be of greater physiological relevance than those isolated during chronic infection (Joseph et al. 2015). They also exhibit shorter and less-glycosylated V1–V4 gp120 domains than chronic HIV-1 strains (Joseph et al. 2015). Glycosylation patterns on TF viral Envs may also be differentiated from those on chronic viral Envs, with the former showing more high-mannose glycans (Joseph et al. 2015). High levels of mannose-type glycans on TF Envs could thus confer differential sensitivity to CBAs (Hu et al. 2015). This was probed by measuring the sensitivities of 20 TF Env pseudotyped viruses to GNA in TZM-bl adenocarcinoma cells (Hu et al. 2015). GNA displayed potent inhibitory effects against the majority of these (IC_{50} s 0.0013 to 0.216 μ M), with only six showing diminished susceptibilities (IC_{50} s > 0.5 μ M) (Hu et al. 2015). It was most effective against the 700,010,058, A4.4375 and WEAUd15.410.787 pseudotypes (IC_{50} s 0.0013 and 0.0041 μ M, respectively) (Hu et al. 2015).

Transfer of HIV-1 to CD4+ lymphocytes can occur via endocytic uptake and viral fusion (Turville et al. 2005). These in turn proceed via either directional regurgitation (also referred to as infectious transfer *in trans*, I-IT) or through de novo viral production in dendritic cells (DCs) resulting in a second-phase transfer to lymphocytes (also known as infectious second-phase transfer, I-SPT) (Turville et al. 2005). The susceptibilities of both pathways to GNA was examined in monocyte-derived DCs, where its EC_{50} for inhibition of I-IT in HIV (strain Bal) was shown to be 0.017 μ g/ml (Turville et al. 2005). Efficient blockage of I-SPT by contrast was harder to achieve as reflected by an EC_{50} of 2.53 μ g/ml for inhibition of the pathway in the same HIV strain (Turville et al. 2005).

GNA_{maize}, a lectin from *Zea mays* L., Poaceae, displayed a 64% sequence similarity to GNA and was also shown to be a tetrameric protein like the latter (Hoorelbeke et al. 2011). However, whilst GNA was specific for mannose-type glycans, GNA_{maize} only recognized complex *N*-glycans (Hoorelbeke et al. 2011). GNA was shown to be much more effective at inhibiting HIV-1(III_B) and HIV-2(ROD) proliferation in CEM cultures (EC_{50} s 0.007 and 0.008 μ M, respectively) than GNA_{maize} (EC_{50} s 0.46 and 0.83 μ M, respectively) (Hoorelbeke et al. 2011). Furthermore, inhibition of syncytium formation between HIV-1-infected HuT-78 lymphocytes and uninfected Sup-T1 lymphoblasts occurred more efficiently with GNA (EC_{50} 0.062 μ M) than with GNA_{maize} (EC_{50} > 1.67 μ M) (Hoorelbeke et al. 2011). Furthermore, GNA demonstrated

better inhibitory effects than GNA_{maize} against DC-SIGN-mediated capture of HIV-1 by DC-SIGN + Raji cells (EC_{50} s 0.04 and 0.90 μ M, respectively) and subsequent transmission to CD4 + T cells (EC_{50} s 0.006 and 0.44 μ M, respectively) (Hoorelbeke et al. 2011). Whilst GNA preferentially selected for strains with deleted mannose-type glycans on gp120, prolonged exposure of HIV-1 to escalating concentrations of GNA_{maize} selected for mutants in which only one complex-type glycan of gp120 was deleted (Hoorelbeke et al. 2011). Thus, the diminished anti-HIV activity of GNA_{maize} compared to GNA may be explained by the shift in glycan recognition and the deletion of carbohydrate-binding sites that have high affinity for mannose oligomers (Hoorelbeke et al. 2011).

With MT-4 cells as host, GNA (EC_{50} 0.8 μ g/ml) was more active than DS-10000 (EC_{50} 1.2 μ g/ml) against SIV infectivity (Balzarini et al. 1991). It was seen to be far less effective on other retroviruses such as SRV (Simian AIDS-related virus) (EC_{50} 100 μ g/ml) and MSV (murine sarcoma virus) (EC_{50} > 100 μ g/ml) that were grown on human Raji lymphoblasts and C3H/3T3 mouse fibroblasts, respectively (Balzarini et al. 1991). GNA was potently active against FIV (feline immunodeficiency virus) (Petaluma) in CRFK cell cultures (EC_{50} 0.09 μ g/ml), over 25 times more so than tenofovir (EC_{50} 2.3 μ g/ml) (Balzarini et al. 2004a). Syncytium formation between SIV(mac251)-infected Raji/DC-SIGN and uninfected C8166 lymphoblasts was strongly inhibited by GNA (IC_{50} 0.046 μ M), to a far greater extent than by pradimicin-A (IC_{50} 19 μ M) (Francois et al. 2008). Mutant SIV(mac251) strains produced by culturing on MT-4 cells with increasing pradimicin-A amounts were characterized by mutations on the gp120 envelope (Francois et al. 2008). One such mutant (PRM-A-1), which reflected changes at amino acid positions Ile248 and Ser462, was shown to be susceptible to GNA (EC_{50} 0.027 μ M) but to a lesser extent than the wild-type strain (EC_{50} 0.006 μ M) (Francois et al. 2008). Eleven of the twenty-four *N*-glycosylation motifs on the HIV-1 gp120 comprise high mannose or hybrid-type sugars, with the remaining thirteen shown to be complex glycans (Leonard et al. 1990). Although SIV also contains twenty-four *N*-glycosylation motifs, the nature of its glycans has not been defined (Chen et al. 2005). GNA (at 1, 10 and 100 μ g/ml) delayed by 8 days the peak for viral replication of SIV(mac239) (from rhesus macaques) in CEMx174 cells (Stansell and Desrosiers 2010). The passage of SIV(mac239) through increasing concentrations of GNA (0.1 to 200 μ g/ml) yielded a resistant population, wherein three sites (of 26 in total) in the envelope protein for *N*-linked carbohydrate attachment (Asn-X-Ser or Asn-X-Thr) were eliminated (Stansell and Desrosiers 2010). Two (Asn244 and Asn460) were in the gp120 surface subunit, whilst the other (Asn625) was in the envelope gp41 transmembrane protein (Stansell and Desrosiers 2010). Furthermore, the variant gp120

exhibited alower capacity for binding to GNA compared to mac239 gp120 (Stansell and Desrosiers 2010).

With Huh-7 hepatocytes as host, GNA exhibited potent effects against hepatitis C virus (HCV) (Flaviviridae) (EC_{50} 0.007 μ M) (Bertaux et al. 2007). The result was amplified by the fact that its effect on the host was minimal (CC_{50} > 10 μ M), as well as the fact that the antiviral activity of DS-5000 was poor (EC_{50} > 50 μ M) (Bertaux et al. 2007). As noted for GNA against HIV-1(III_B) above, there was also a pronounced decline in its anti-HCV activity (EC_{50} 0.15 μ M) with the introduction of mannan (2.5 mg/ml) to culture cells (Bertaux et al. 2007). Furthermore, the entry of HCV pseudoparticles (HCVpp) into Huh-7 cells was effectively blocked by GNA (EC_{50} 0.0009 μ M) (Bertaux et al. 2007). DC-SIGN is an important binding receptor for dengue virus (DENV) (Flaviviridae), where it recognizes *N*-glycosylation sites on the viral E-glycoprotein (Lozach et al. 2005). Whilst the virus cannot infect the human B cell line Raji/0, it can do so with DC-SIGN-expressing Raji cells (Alen et al. 2009). IL-4 (interleukin-4)-treated monocytes, which express DC-SIGN on their surface, are likewise infected by dengue virus (Alen et al. 2009). GNA was able to remarkably stifle infection in both of these DC-SIGN-expressing systems (EC_{50} s 0.21 and 0.0032 μ M, respectively) without detriment to the attendant host cells (CC_{50} s > 5000 μ M in each) (Alen et al. 2009). The infection of monkey kidney epithelial (Vero) cells (devoid of DC-SIGN), which are naturally susceptible to the virus, was also not affected by GNA (EC_{50} > 5000 μ M) (Alen et al. 2009). This suggested that the entry mechanisms for the virus must be dependent on cell type (Alen et al. 2009). The four serotypes of dengue virus DENV-1, DENV-2, DENV-3 and DENV-4 were all highly susceptible to GNA in monocyte-derived dendritic cells (MDDCs) (EC_{50} s 0.022, 0.0038, 0.011, 0.0056 μ M, respectively) (Alen et al. 2011). There was a sharp drop-off for GNA when Raji/DC-SIGN cells were used as hosts (EC_{50} s 0.28, 0.24, 0.17, 0.56 μ M, respectively) (Alen et al. 2011). The carbohydrate-binding antibiotic pradimicin-S dose-dependently inhibited DENV-2 replication in MDDCs (EC_{50} 11.0 μ M), but was ineffective when Raji/DC-SIGN cells were utilized as host (EC_{50} > 55 μ M) (Alen et al. 2011). Since DCs exist in an immature state in most tissue, they lack the expression of CD40 and CD86 and are thus unable to stimulate T cells (Banchereau and Steinman 1998). They are, however, equipped with the requisite receptors (such as DC-SIGN) that are pivotal in the attachment and capture of diverse pathogens (Banchereau and Steinman 1998). Immature DC-SIGN-expressing MDDCs used in the study of Alen et al. (2011) were generated out of primary monocytes (PBMCs). It was also demonstrated that GNA acted at an early stage of DENV-2 infection of Raji/DC-SIGN cells, binding to the viral envelope thereby impeding viral attachment (Alen et al. 2011). Furthermore,

weak activity for GNA was observed when it was administered after the virus attachment step (Alen et al. 2011). GNA was also able to prevent the cellular activation and differentiation process of MDDC induced by DENV-2 infection (Alen et al. 2011). With the inclusion of GNA (2.3 μ M) in DENV-2-infected MDDCs, the levels of CD80, CD86 and DC-SIGN were almost identical to the levels seen in uninfected immature cell cultures (Alen et al. 2011).

Galanthus nivalis agglutinin also inhibited human cytomegalovirus (CMV) (Herpesviridae) replication in human erythroleukaemia HEL cells with EC_{50} s of 0.9 and 1.6 μ g/ml against the AD169 and Davis strains, respectively (Balzarini et al. 1991). The reference standard 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG) reflected EC_{50} values of 0.8 μ g/ml, respectively (Balzarini et al. 1991). The responses of GNA and DHPG were highly selective given that their respective minimum toxic concentrations (MTCs) were > 100 μ g/ml (Balzarini et al. 1991). A further pathogen of the Herpesviridae to be exposed to GNA was the herpes simplex virus, HSV-1 (herpes simplex virus-1) and HSV-2 strains of which (relative to DS-5000, mean EC_{50} 0.8 μ M) proved to be moderately resistant to the lectin (EC_{50} s > 1 μ M) (Bertaux et al. 2007). Furthermore, the effects of GNA on the host HEL cells were mild (CC_{50} > 2 μ M) (Bertaux et al. 2007).

Galanthus nivalis agglutinin was examined for effects on vesicular stomatitis virus (VSV) of the Rhabdoviridae against which its effect was mild (EC_{50} > 2 μ M) compared to that of DS-5000 (EC_{50} 0.8 μ M), also proving to be ineffective on host HeLa adenocarcinoma cultures (CC_{50} > 2 μ M) (Bertaux et al. 2007). The effects of GNA and DS-5000 on respiratory syncytial virus (RSV) (Pneumoviridae) were the same as those seen with VSV (EC_{50} s > 2 and 0.8 μ M, respectively) (Bertaux et al. 2007). Likewise, its CC_{50} against HeLa cells was determined to be > 2 μ M (Bertaux et al. 2007). GNA has also been exposed to the parainfluenza-3 virus (Paramyxoviridae), where its effect was poor against both the virus and host Vero cells (EC_{50} , CC_{50} s > 2 μ M, respectively) (Bertaux et al. 2007). DS-5000 by comparison proved to be far less effective on this respiratory virus (EC_{50} > 100 μ M) (Bertaux et al. 2007).

Severe acute respiratory syndrome-coronavirus (SARS-CoV) (Frankfurt 1 strain) and feline infectious peritonitis virus (FIPV) strain 79–1146 were the two members of the Coronaviridae that were examined for infectivity of monkey kidney Vero and Crandell-Rees feline kidney (CRFK) cells, respectively (Keyaerts et al. 2007). The effect was marked by low cytotoxicities of GNA towards the host cells (CC_{50} s > 100 μ g/ml) as well as moderate activities against the viruses (EC_{50} s 6.2 and 3.9 μ g/ml, respectively) (Table S2) (Keyaerts et al. 2007). Further investigation of FIPV involved the TN406HP strain infection of FCWF (*Felis catus* whole fetus) cells, against which GNA was

remarkably active (EC_{50} 0.008 μ M) relative to pradimicin-A (EC_{50} 2.5 μ M) (Van der Meer et al. 2007a). A 16-fold depreciation in activity for GNA was observed when screening was switched to the 79–1146 strain of FIPV (EC_{50} 0.13 μ M) (Van der Meer et al. 2007a). GNA (at 50 μ g/ml) also reduced syncytium formation in 79–1146 infected FCWF cells compared to untreated cells (Van der Meer et al. 2007b). At the same concentration, GNA also inhibited syncytium formation in mouse hepatitis virus (MHV) (strain A59) infected mouse LR7 fibroblast cells (Van der Meer et al. 2007b). From this it was suggested that the antiviral effect of GNA may be due to it binding the coronavirus S-protein, the expression of which on the cell surface signals the onset of cell fusion and syncytium formation (Van der Meer et al. 2007b). Three mutant strains of MHV (Alb248, Alb138 and Alb244) were utilized to gauge the effects of GNA on the coronavirus M-protein (Van der Meer et al. 2007b). These were characterized by their abilities to express M-proteins with no sugar moieties, O-linked sugars and N-linked sugars, respectively (Van der Meer et al. 2007b). The best activity for GNA was against the Alb248 strain (EC_{50} 0.4 μ g/ml), falling off considerably for the other two mutants (EC_{50} s 1.8 and 3.7 μ g/ml, respectively) (Van der Meer et al. 2007b). The reference standard pradimicin-A exhibited EC_{50} values of 2.0, 5.7 and 4.1 μ g/ml, respectively (Van der Meer et al. 2007b). From the results, it was suggested that besides the S-protein, the M-protein may also be considered as an additional antiviral target for GNA (Van der Meer et al. 2007b). Feline coronavirus (FCoV) strain 79–1683 infectivity of FCWF cells was also negated by GNA (EC_{50} 0.43 μ M) to a greater extent than by pradimicin-A (EC_{50} > 120 μ M) (Van der Meer et al. 2007a). GNA and nelfinavir were the only two compounds (out of sixteen tested) that prevented FCoV(NTU156) infection of FCWF cells, with IC_{50} s of 8.80 and 8.19 μ M respectively (Hsieh et al. 2010). The library was composed of commercial antiviral drugs that were classified as to whether they were nucleoside analogues (such as acyclovir), protease inhibitors (such as indinavir) or reverse transcriptase inhibitors (such as nevirapine) (Hsieh et al. 2010). GNA and nelfinavir lost their efficacies when the viral load was increased from 2000 to 20,000 plaque forming units per millilitre (PFU/ml) to mimic the high loads associated with infected animals (Hsieh et al. 2010). The high load deficiency could nevertheless be overcome when both compounds were used in tandem (Hsieh et al. 2010). The possibility of the antiviral effect being manifested via cytotoxicity was ruled out since the compounds, individually or in combination, had no detrimental effects on the host FCWF cells (Hsieh et al. 2010). Infection of Vero cells by avian infectious bronchitis virus (IBV) (strain Beaudette) was strongly inhibited by GNA (EC_{50} 0.0002 μ M), orders of magnitude more so than pradimicin-A (EC_{50} 2.9 μ M) (Van der Meer et al. 2007a). GNA also prevented (EC_{50}

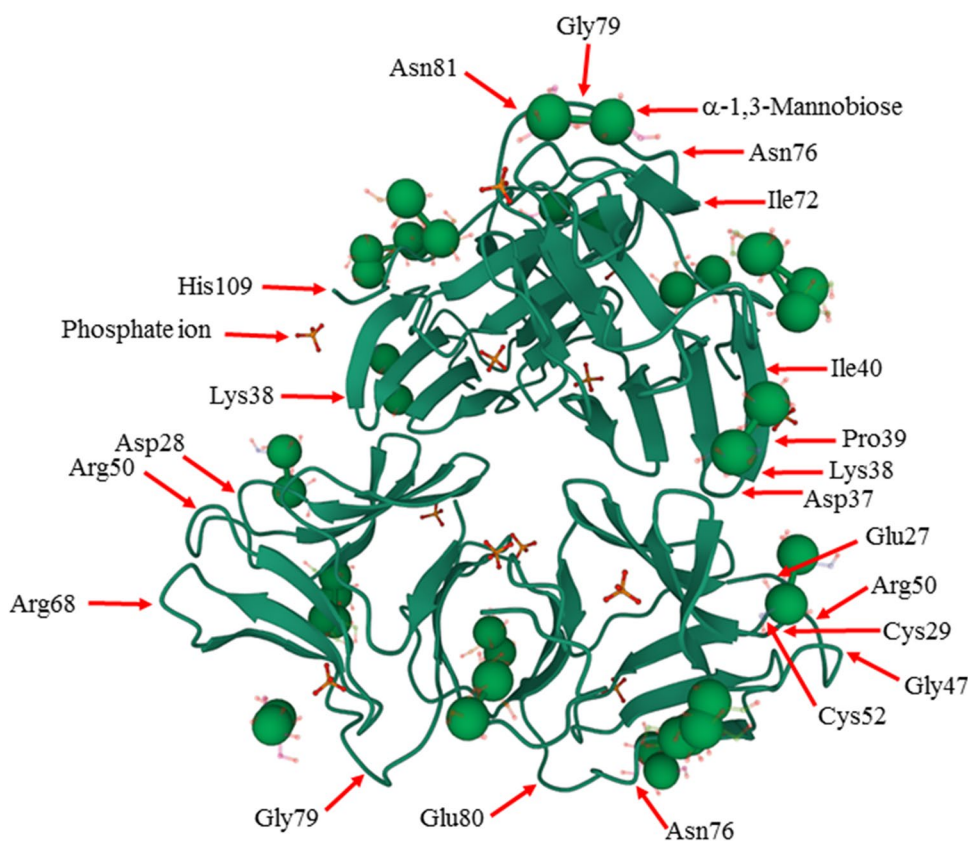
0.004 μ M) transmissible gastroenteritis virus TGEV (strain Purdue) from infecting swine testicular (ST) cells by a factor of over 1000 compared to pradimicin-A (EC_{50} 4.7 μ M) (Van der Meer et al. 2007a).

The equine pathogen Berne virus (family Tobnaviridae) also succumbed to GNA exposure in equine dermis (Ederm) cells (EC_{50} 0.12 μ M), at a markedly lower level than that demonstrated for pradimicin-A (EC_{50} 31 μ M) (Van der Meer et al. 2007a). A further equine virus screened was the equine arteritis virus (EAV) (Arteriviridae) against which GNA (in equine dermis cells) proved to be far more potent than pradimicin-A (EC_{50} s 0.18 and > 120 μ M, respectively) (Van der Meer et al. 2007a).

***Narcissus pseudonarcissus* Agglutinin**

Narcissus pseudonarcissus agglutinin (NPA) was described not long after GNA and shown to reflect several similarities to the latter, both in terms of its structure and activity (Van Damme et al. 1988). It was serologically identical to GNA and structurally composed of the same 13-kDa subunits that lacked disulphide bridging, the difference being that whereas GNA was a tetramer NPA was arranged into a dimeric structure (Van Damme et al. 1988). Small-angle X-ray solution scattering later revealed that it can also exist as a tetramer in solution (Sauerborn et al. 1999). X-ray crystallographic analysis of the NPA/ α -1,3-mannobiose complex (Fig. 3) indicated that the key structural feature of the lectin was the presence of three fully occupied binding pockets per monomer, arranged around the faces of a triangular β -prism motif (Sauerborn et al. 1999). It also displayed mannose-binding specificity and readily agglutinated rabbit erythrocytes exposed to trypsin, without having any effects on human RBCs (Van Damme et al. 1988). NPA also displayed notable specificity for α (1,3)-linked mannose residues (Van Damme et al. 1988). The same seventeen amino acids were detected in NPA, with similar (but not identical) levels to those seen for GNA (Van Damme et al. 1988). NPA exhibited mild activities against HIV-1(III_B) (Retroviridae) when culturing was carried out in MT-2 (IC_{50} 4.97 μ g/ml), CEM lymphoblast (IC_{50} 2.18 μ g/ml) and U937 myeloid leukaemia (IC_{50} 7.31 μ g/ml) cells, respectively (Table S1) (Weiler et al. 1990). Similarly mild activities were observed against HIV-2(ST) and HIV-2(MS) in CEM and U937 cells (IC_{50} s 6.58 and 8.57 μ g/ml, respectively) (Weiler et al. 1990). By comparison, the mean IC_{50} value determined for AZT in these five instances was 0.043 μ g/ml (Weiler et al. 1990). Nonetheless, NPA exhibited negligible cytotoxic effects on all three host cell lines as TC_{50} values in all cases were > 100 μ g/ml (Weiler et al. 1990). It dose-dependently (0.3, 1.0, 3.0 μ g/ml) inhibited syncytium formation between Jurkat lymphocytic leukaemia cells and HIV-1 producing H9 lymphocytic cells, with complete inhibition observed at the highest tested

Fig. 3 Crystal structure of NPA (resolved at 2.0 Å) in complex with α -1,3-mannobiose, where asparagine/aspartic acid and glycine constituted over 25% of the amino acid residues. Structure downloaded free of charge from the Protein Data Bank using the PDB <http://doi.org/10.2210/pdb1NPL/pdb>; see also Sauerborn et al. (1999)



concentration (Weiler et al. 1990). NPA (at 20 $\mu\text{g/ml}$) also displayed the ability to inhibit the binding of HIV-1 to MT-2 cells in the same way that the antibody OKT4A (at 100 $\mu\text{g/ml}$) was able to (Weiler et al. 1990). This was significant since OKT4A functions by preventing HIV binding to CD4 receptors on cells (Weiler et al. 1990). In the same study, sulphoevernan, a polyanionic polysaccharide, displayed potent effects against HIV-1 infection of MT-2 cells (IC_{50} 0.52 $\mu\text{g/ml}$) (Weiler et al. 1990). A competing experiment with NPA was carried out to ascertain whether sulphoevernan protected MT-2 cells by binding to receptors on the virus (Weiler et al. 1990). It was seen that pre-incubation of virus particles with NPA did markedly prevent binding of sulphoevernan to the virus (Weiler et al. 1990). The rationale for this was that NPA, with its affinity for $\alpha(1,3)$ -linked mannose residues, would be able to mask the carbohydrate structures of the viral transmembrane envelope protein gp20 (Weiler et al. 1990).

Narcissus pseudonarcissus agglutinin displayed good activities against HIV-1(III_B) and HIV-2(LAV-2) infection of MT-4 cells (EC_{50} s 0.6 $\mu\text{g/ml}$, respectively) relative to DS-10000 (EC_{50} 0.4 $\mu\text{g/ml}$) (Balzarini et al. 1991). Syncytium formation between HIV-1 or HIV-2 infected HuT-78 lymphocytes and uninfected Molt-4 lymphoblasts was suppressed to a greater extent by NPA (EC_{50} s 3.3 and 4.0 $\mu\text{g/ml}$, respectively) than by DS-5000 (EC_{50} 25 $\mu\text{g/ml}$) (Balzarini

et al. 1991). NPA exhibited weak activity against anti-gp120 binding to HuT-78/HIV-1 cells (12% inhibition after 15 min) and did not inhibit OKT4A binding to MT-4 cells (Balzarini et al. 1991). NPA only blocked HIV-1 adsorption by MT-4 cells at higher concentrations (> 100 $\mu\text{g/ml}$), compared to DS-5000 which caused complete blockage at 25 $\mu\text{g/ml}$, suggesting that it did not markedly inhibit HIV-1 binding to CD4+ cells (Balzarini et al. 1991).

Capture of HIV-1 particles by DC-SIGN-expressing B-lymphoblastic Raji cells was inhibited by NPA (IC_{50} 0.7 $\mu\text{g/ml}$), much more than by AMD-3100 or DS-5000 (IC_{50} s > 50 and > 250 $\mu\text{g/ml}$, respectively) (Balzarini et al. 2007). Wild-type Raji/0 cells by contrast were noted for their inability to effect such a capture (Balzarini et al. 2007). Furthermore, syncytium formation in HIV-1(III_B)-infected C8166 CD4+ T lymphocytes as well as between Raji/DC-SIGN and uninfected C8166 cells was inhibited by NPA (EC_{50} s 0.9 and 3.1 $\mu\text{g/ml}$, respectively) (Balzarini et al. 2007). However, the effect was more pronounced when AMD-3100 (mean EC_{50} 0.44 $\mu\text{g/ml}$) or DS-500 (mean EC_{50} 2.80 $\mu\text{g/ml}$) were used as inhibitors (Balzarini et al. 2007).

Narcissus pseudonarcissus agglutinin is able to target the glycans of HIV-1 gp120, thereby preventing DC-SIGN capture of the virus by dendritic cells and subsequent transmission to CD4+ T lymphocytes (Pollicita et al. 2008). The infection of human primary monocyte-derived macrophages

(MDMs) by HIV-1 (BaL) was efficiently prevented by NPA (EC_{50} 0.026 μ M) relative to enfuvirtide (EC_{50} 0.020 μ M) (Pollicita et al. 2008). Both R5 and X4 HIV-1 strains (III_B, NL4.3, MN, HE, RF, BaL) were readily captured by the macrophage mannose-binding receptor (MMR) present on MDMs (Pollicita et al. 2008). In a similar fashion, virus capture was also demonstrated with Raji/DC-SIGN B-lymphocytes (Pollicita et al. 2008). Nonetheless, NPA was shown to inhibit HIV-1 (NL4.3) capture by MMR-expressing MDMs (IC_{50} 0.4 μ M) in a similar fashion that the MMR antibody was able to (Pollicita et al. 2008). Syncytium formation in cocultures of HIV-1-captured MDMs and uninfected C8166 CD4 + T lymphocytes was also inhibited by NPA at its half-maximal inhibitory concentration (Pollicita et al. 2008). Antigen-presenting cells (APCs) such as MDMs and DCs are major sites for viral infection and are thus crucial for activating the immune system (Banchereau et al. 2001). They present antigens for stimulation of primary and secondary T cell responses by MHC (major histocompatibility complex) class I and II pathways and are also known to express the CD4 receptor (Chougnnet et al. 2002).

The HIV-1(III_B) mutant strains GNA-1.0, GNA-1.1a and GNA-1.2 produced from variable GNA concentrations as described above were also exposed to NPA in CEM cells (Balzarini et al. 2004b). Its respective EC_{50} values were determined to be 0.90, 30 and 43.3 μ g/ml, relative to tenofovir (mean EC_{50} 5.3 μ g/ml) (Balzarini et al. 2004b). The wild-type HIV-1(III_B) variant proved to be much more susceptible to NPA (EC_{50} 0.40 μ g/ml) compared to tenofovir (EC_{50} 3.3 μ g/ml) (Balzarini et al. 2004b). Furthermore, syncytium formation between infected HuT-78 (or CEM) cells and uninfected Sup-T1 cells was inhibited by NPA to a greater extent in the wild-type strain (mean EC_{50} 4.6 μ g/ml) than in the GNA-2.1 mutant (mean EC_{50} 30.4 μ g/ml) (Balzarini et al. 2004b). The HIV-1(III_B) mutant GNA-500CS generated from variable GNA doses (as described above) was also exposed to NPA in CEM cells (Balzarini et al. 2005a). It proved to be highly resistant to NPA (EC_{50} > 100 μ g/ml), much more than the wild-type HIV-1(III_B) (EC_{50} 0.37 μ g/ml) (Balzarini et al. 2005a). Seven HIV-1(III_B) strains identified after exposure to the agglutinin (UDA) from *Urtica dioica* L., Urticaceae, in CEM cells were characterized by changes to amino acids at gp120 of the viral glycoprotein as outlined above (Balzarini et al. 2005b). NPA was much more effective on wild-type HIV-1(III_B) (EC_{50} 0.009 μ M) than it was against these mutant strains (mean EC_{50} 0.30 μ M) (Balzarini et al. 2005b). DS-5000 was less active than NPA in both instances (EC_{50} 0.08 μ M, mean EC_{50} 0.15 μ M), but similar in selectivity towards the wild-type strain (Balzarini et al. 2005b). Its activity against SIV(mac251) (EC_{50} 0.5 μ g/ml) in MT-4 cells was even better than that of DS-10000 (EC_{50} 1.2 μ g/ml) (Balzarini et al. 1991). However, it was ineffective against the two remaining Retroviridae members MSV

(EC_{50} 60 μ g/ml in C3H/3T3 cells) and SRV (EC_{50} > 200 μ g/ml in Raji cells) (Balzarini et al. 1991).

Narcissus pseudonarcissus agglutinin was also active against CMV (of the Herpesviridae) in HEL cells with respective EC_{50} s of 1.2 and 1.6 μ g/ml against the AD169 and Davis strains that were roughly twice those seen for DHPG (Balzarini et al. 1991). MTCs for both entities against host cells were > 100 μ g/ml indicating that they were highly selective (Balzarini et al. 1991). The effects of NPA on SARS-CoV (Frankfurt 1) and FIPV (79–1146) (Coronaviridae) were probed in monkey kidney Vero and Crandell-Rees feline kidney cells, respectively, where it performed better against the former (EC_{50} 5.7 μ g/ml) than the latter (EC_{50} 24 μ g/ml) (Table S2) (Keyaerts et al. 2007). Accompanying the antiviral effects were markedly low cytotoxicities manifested on host cells (CC_{50} s > 100 μ g/ml, respectively) (Keyaerts et al. 2007).

***Narcissus lobularis* Agglutinin**

Narcissus lobularis agglutinin (NLA) was also composed of a 13-kDa subunit which by electrophoresis separated into two closely migrating bands, suggesting a dimeric form for its structure (Animashaun et al. 1993). Examined for effects against HIV-1(III_B) infectivity, it was shown that pre-incubation of the virus with NLA (at 100 μ g/ml) inhibited (relative to AZT) syncytium formation in C8166 T cell leukaemia host cells, without it being effective at lower concentrations (1 and 10 μ g/ml, respectively) (Animashaun et al. 1993). Furthermore, NLA caused dose-dependent depletion of the virus, with no viral progeny detectable at the highest tested concentration (100 μ g/ml) (Animashaun et al. 1993). In terms of cell viability, uninfected C8166 cells remained fully viable at all three tested dosages (1, 10 and 100 μ g/ml) (Animashaun et al. 1993). Infected cells by contrast were only fully viable following exposure to NLA at 100 μ g/ml, dropping to 58% and 33% with the two remaining concentrations, respectively (Animashaun et al. 1993).

***Narcissus tazetta* Agglutinin**

Narcissus tazetta agglutinin (NTA) was isolated from the ‘Chinese daffodil’ *Narcissus tazetta* var. *chinensis* L. (Ooi et al. 1998). It exhibited a molecular mass of 26 kDa by gel filtration and 13 kDa by SDS-PAGE, suggesting it to be a dimer with two identical subunits (Ooi et al. 1998). cDNA amino acid sequencing revealed that NTA contained a mature polypeptide consisting of 105 amino acids and a C-terminal peptide extension (Ooi et al. 2010). A 3D model showed that the primary polypeptide contained three subdomains, each with a conserved mannose-binding site (Ooi et al. 2010). NTA showed high homology (60–80% similarity) with other Amaryllidaceae mannose-binding lectins,

such as GNA, NPA and HHA (Ooi et al. 2010). Whilst NTA (unlike NPA) did not agglutinate rabbit erythrocytes, it did inhibit protein synthesis in rabbit reticulocytes (50% at 7.1 µg/ml) (Ooi et al. 2008). Examined for antiviral effects, it moderately inhibited influenza A (H1N1) virus (Orthomyxoviridae) multiplication in Madin-Darby canine kidney (MDCK) cells (EC_{50} 4.50 µg/ml) relative to ribavirin (Ooi et al. 2008). H1N1 and H3N2 proved to be much more susceptible to NTA (IC_{50} s 1.33 and 0.4 µg/ml, respectively) than the H5N1 strain (IC_{50} > 20 µg/ml) (Ooi et al. 2010). These effects proceeded without significant reduction in the viability of MDCK host cells in the dosage range 0.05–500 µg/ml (Ooi et al. 2010). The influenza B virus (HongKong/CUHK-24964/2004), however, was shown to be most susceptible to NTA (IC_{50} 0.2 µg/ml) (Ooi et al. 2010). Time of addition studies using the haemagglutinin assay showed that the antiviral activity of NTA was more pronounced at the early stage of the H1N1 viral cycle (Ooi et al. 2010).

A further pathogen examined was the respiratory syncytial virus (RSV) (Pneumoviridae), whose growth in HEP-2 human epithelial cells NTA inhibited by 38% (at 50 µg/ml) (Ooi et al. 2008). Plaque formation by RSV was also dose-dependently inhibited by NTA (IC_{50} 2.3 µg/ml) in the self same host, with a selectivity index (SI) (141.4) that largely favoured the antiviral response (Ooi et al. 2008). Time of addition studies suggested that NTA was effective during the whole RSV infection cycle (Ooi et al. 2008). As such, the plaque inhibition rate was 76.4% when NTA was present during the whole infection cycle, 36.6% when present only during the first 2 h of infection and 45.7% if it was added 2 h post-infection (Ooi et al. 2008). A preliminary scan of NTA for anti-herpes activity showed a positive response for the lectin against HSV-1 (Herpesviridae) (Ooi et al. 2014).

Other *Narcissus* Agglutinins

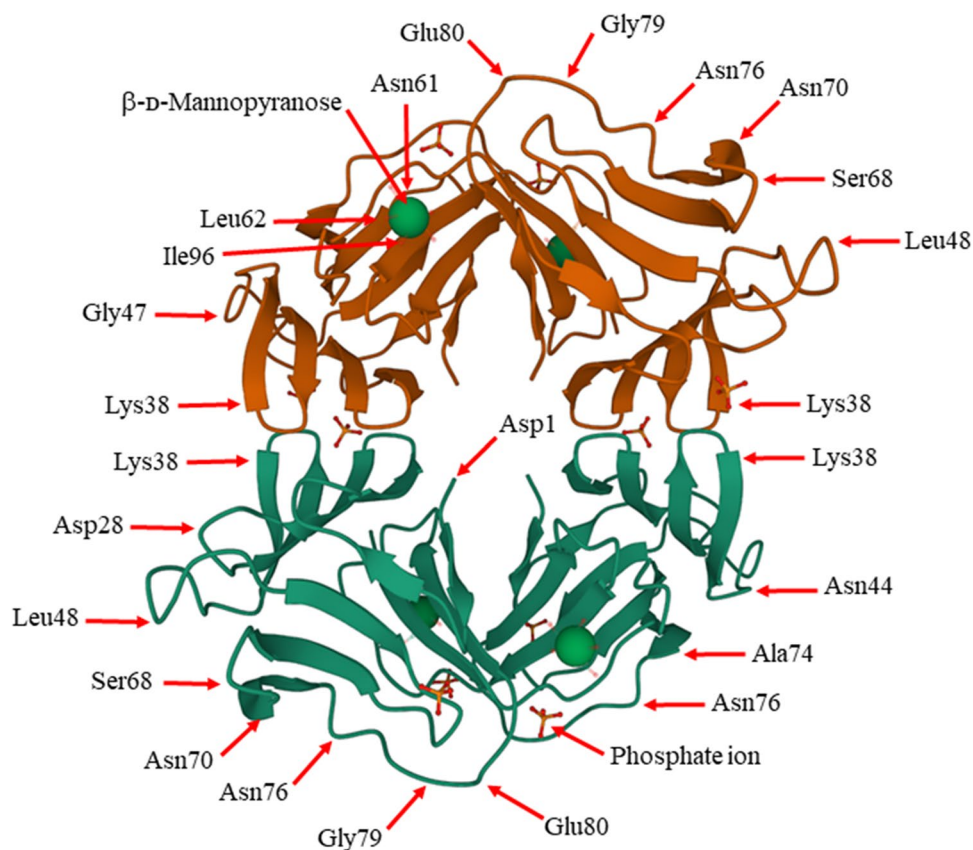
Several other lectins were described from *Narcissus* species, all of which exhibited a common molecular weight value of 37.5 kDa (Lopez et al. 2003). Those with notable activities against HIV-1(NL4.3) infection of MT-4 cells included *N. tortifolius* agglutinin (EC_{50} 0.056 µg/ml, CC_{50} 109.3 µg/ml), *N. abscissus* agglutinin (EC_{50} 0.91 µg/ml, CC_{50} > 125 µg/ml), *N. confusus* agglutinin (EC_{50} 0.30 µg/ml, CC_{50} 106 µg/ml), *N. leonensis* agglutinin (EC_{50} 0.17 µg/ml, CC_{50} 78.9 µg/ml) and *N. perez-chiscanoi* agglutinin (EC_{50} 0.66 µg/ml, CC_{50} > 125 µg/ml) (Lopez et al. 2003). The reference standards used in these instances were dextran sulphate (EC_{50} 0.54 µg/ml, CC_{50} > 125 µg/ml) and AZT (EC_{50} 0.009 µg/ml, CC_{50} > 2 µg/ml) (Lopez et al. 2003). Furthermore, there were no correlations between anti-HIV and haemagglutination activities for any of the lectins, from which it was suggested that this could be a consequence of their multiple isolectin compositions (Lopez et al. 2003).

Hippeastrum Hybrid Agglutinin

Hippeastrum sp. hybrid agglutinin (HHA), like GNA, was shown to be a tetramer of the 13-kDa subunits (Balzarini et al. 1991). However, whilst GNA had a specificity for $\alpha(1,3)$ -linked mannose residues, HHA recognized both $\alpha(1,3)$ - and $\alpha(1,6)$ -linked mannose residues (Balzarini et al. 1991). X-ray crystallographic analysis of the HHA/ β -D-mannopyranose complex showed that there were two protein molecules in the asymmetric unit with 109 amino acid residues per monomer (Fig. 4) (Chantalat et al. 1996). Similar to the GNA and NPA lectins, HHA markedly inhibited HIV-1(III_B) and HIV-2(LAV-2) (Retroviridae) infection of MT-4 cells (EC_{50} s 0.4 µg/ml, respectively) (Table S1), proving to be equally as effective as the sulphated polysaccharide DS-10000 (Balzarini et al. 1991). Its action against HIV was shown to involve (i) suppression of syncytium formation between HIV-1- or HIV-2-infected HuT-78 lymphocytes and uninfected Molt-4 lymphoblasts (EC_{50} s 2.0 µg/ml, respectively), (ii) moderate efficacy against anti-gp120 binding HuT-78/HIV-1 cells (45% inhibition after 15 min), (iii) no inhibition against OKT4A binding MT-4 cells and (iv) weak blockage of HIV-1 adsorption by MT-4 cells (Balzarini et al. 1991).

The responses of HHA to drug-resistant HIV strains in CEM cells were similar to those for GNA above (Balzarini et al. 2004a). These included NNRTI-resistant HIV-1 (EC_{50} 0.75 µg/ml), lamivudine-resistant HIV-1 (EC_{50} 0.35 µg/ml), zidovudine-resistant HIV-1 (EC_{50} 0.80 µg/ml) and protease inhibitor-resistant HIV-1 (EC_{50} 0.53 µg/ml), against which tenofovir reflected a mean EC_{50} of 8.45 µg/ml (Balzarini et al. 2004a). The HIV-1(III_B) strain was shown to be highly susceptible to HHA in CEM cells (EC_{50} 0.006 µM), much more so than to DS-5000 (EC_{50} 0.40 µM) (Bertaux et al. 2007). The EC_{50} dropped dramatically to 0.50 µM when mannan (2.5 mg/ml) was included with the culture medium (Bertaux et al. 2007). The performance of DS-5000 (EC_{50} 0.30 µM) was also affected by mannan, but not to the same extent seen with GNA (Bertaux et al. 2007). HHA inhibited HIV-2(strain ROD) (EC_{50} 0.016 µM) relative to DS-5000 (EC_{50} 0.16 µM), the corresponding effects of which were mild on CEM host cells (CC_{50} s > 2 and > 100 µM, respectively) (Bertaux et al. 2007). Virus adsorption inhibitor-resistant (DS-5000) and virus entry inhibitor-resistant (AMD-3100 and SDF-1) HIV-1 strains were also sensitive to HHA in MT-4 cells (EC_{50} s 0.23, 0.24 and 0.55 µg/ml, respectively) (Balzarini et al. 2004a). The wild-type HIV-1 strain (NL4.3) from which the resistant strains were produced also succumbed to HHA (EC_{50} 0.57 µg/ml), but less so than by DS-5000 (EC_{50} 0.14 µg/ml) or AMD-3100 (EC_{50} 0.005 µg/ml) (Balzarini et al. 2004a). It was over 7 times more effective at inhibiting syncytium formation between HIV-1 infected HuT-78 and uninfected lymphoblastic

Fig. 4 Crystal structure of HHA complexed to β -D-mannopyranose (resolved at 2.2 Å) with 109 amino acid residues per monomer. Structure accessed from the Protein Data Bank without charge using the PDB <http://doi.org/10.2210/pdb8A9M/pdb>; see also Chantalat et al. (1996)



leukaemia Sup-T1 cells than it was against the corresponding HIV-2 system (EC_{50} s 1.25 and 9 μ g/ml, respectively), with DS-5000 used as reference (EC_{50} s 2.7 and 10 μ g/ml, respectively) (Balzarini et al. 2004a). HHA (like GNA) had no effect on CD4, CXCR4, CCR5 or DC-SIGN, neither did it exhibit binding affinity for uninfected cell membranes, suggesting that it may affect virus entry by interfering with the virus envelope glycoprotein (Balzarini et al. 2004a). HHA potently inhibited HIV-1(NL4.3) infection of C8166 CD4 + T lymphocytes as determined by flow cytometric analysis (EC_{50} 0.057 μ g/ml) and microscopic scoring (EC_{50} 0.046 μ g/ml) (Petrova et al. 2013). Its effect was shown to be far better than that of pradimicin-A (EC_{50} s 4.4 and 3.9 μ g/ml, respectively) (Petrova et al. 2013). Syncytium formation between infected HuT-78 lymphocytes and uninfected Sup-T1 cells was also inhibited by HHA (EC_{50} 0.27 μ g/ml) (Petrova et al. 2013). It suppressed the capture of viral particles by DC-SIGN + Raji cells in a dose-dependent manner (~50% inhibition at 5.75 μ g/ml, *i.e.* 125 times the EC_{50}) (Petrova et al. 2013). In addition, HHA dose-dependently prevented the transmission of Raji/DC-SIGN-captured viral particles to uninfected C8166 cells (50% inhibition at its EC_{50} value) (Petrova et al. 2013). Synergistic effects were detected for HHA and tenofovir against HIV-1(NL4.3) (Ferir et al. 2011). Individual EC_{50} values against replication in MT-4 cells were found to be 0.022 and 6.3 μ M,

respectively, which were improved to 0.007 and 2.1 μ M following mixing of the compounds at the same concentrations (Ferir et al. 2011). Synergism was also observed during HIV-1(BaL) infection of PBMCs, where EC_{50} s were 0.008 and 0.18 μ M, respectively, when used in a 1:1 ratio (Ferir et al. 2011). These represented notable improvements to the scenario where each compound was evaluated singly (EC_{50} s 0.08 and 0.30 μ M, respectively) (Ferir et al. 2011).

Analysis of p24 expression indicated significant capture of virus particles following exposure of HIV-1 to DC-SIGN-expressing B-lymphoblastic Raji cells (Raji/DC-SIGN), a process that could be inhibited by HHA (IC_{50} 8.5 μ g/ml) many times more than by AMD-3100 or DS-5000 (IC_{50} s > 50 and > 250 μ g/ml, respectively) (Balzarini et al. 2007). The wild-type Raji/0 variant of the lymphoblasts by contrast was noted for its inability to effect such a capture (Balzarini et al. 2007). Examined for its effect on syncytium formation in HIV-1(III_B)-infected C8166 CD4 + T lymphocytes and between Raji/DC-SIGN and uninfected C8166 cells, HHA (EC_{50} s 13 and > 20 μ g/ml, respectively) proved to be far less efficacious than AMD-3100 (mean EC_{50} 0.44 μ g/ml) and DS-500 (mean EC_{50} 2.80 μ g/ml) (Balzarini et al. 2007). Thus, the potential of CBAs to hinder DC-SIGN-expressing cells from transmitting HIV to T lymphocytes could be an important characteristic to consider in microbicidal drug development (Balzarini et al. 2007).

Human embryonic kidney 293 T-REx cells expressing either DC-SIGN or L-SIGN (via doxycycline exposure) were highly susceptible to HIV-1 strain NL4.3-EGFP (Auwerx et al. 2009). Nonetheless, viral capture was effectively prohibited by HHA (EC_{50} s 0.022 and 0.014 μ M, respectively) relative to pradimicin-A (EC_{50} s 35 and 11 μ M, respectively) and DS-5000 (EC_{50} s > 250 and 37 μ M, respectively) (Auwerx et al. 2009). A further aspect to its reactivity was the fact that HHA inhibited dose-dependently syncytium formation between HIV-1-captured 293 T-REx cells (expressing DC- or L-SIGN) and uninfected C8166 CD4 + T lymphocytes (Auwerx et al. 2009). These observations suggested that Amaryllidaceae lectins may have significant potential in drug development as targets for HIV entry and fusion (Auwerx et al. 2009).

A variety of HIV-1(III_B) strains were identified after culturing with increasing concentrations of HHA (1, 10, 20 and 100 μ g/ml) including HHA-1.1, HHA-1.3, HHA-2.1 and HHA-2.2, respectively (Balzarini et al. 2004b). These mutants displayed diminishing sensitivities to HHA in CEM cells (EC_{50} s 2.0, 11.0, 6.1 and 18.8 μ g/ml, respectively) compared to the wild-type strain (EC_{50} 0.28 μ g/ml) (Balzarini et al. 2004b). Tenofovir was active across all five strains with a mean EC_{50} value of 3.4 μ g/ml (Balzarini et al. 2004b). Amino acid discrepancies in the mutants were shown to occur at *N*-glycosylation sites of the envelope glycoprotein gp120 and not in gp41 (Balzarini et al. 2004b). HHA was effective against wild-type HIV-1(BRU) (EC_{50} 0.23 μ g/ml), around 20 times more so than its effect on a clone that was mutated at some *N*-glycosylation sites around the LA loop of gp120 (Balzarini et al. 2004b). The HIV-1(III_B) mutants GNA-500CS, HHA-500CS and HHA-500SN produced from GNA and HHA, respectively, showed cross resistance to HHA in CEM cells (EC_{50} s 103, 55 and 193 μ g/ml) (Balzarini et al. 2005a). This was opposite to the effect observed for HHA against wild-type HIV-1(III_B) (EC_{50} 0.21 μ g/ml) (Balzarini et al. 2005a). HHA-500CS and HHA-500SN (like GNA-500CS above) were resistant to the mannose-binding entities Mab 2G12 and cyanovirin-N, but susceptible to HIV entry inhibitors DS-5000, AMD-3100 and T-20 as well as to HIV reverse transcriptase inhibitors UC-781 and tenofovir (Balzarini et al. 2005a). Further insights to the HIV-1(III_B) mutants HHA-500CS and HHA-500SN were afforded via studies undertaken in conjunction with the α (1,2)-mannosidase I inhibitor DMJ (Balzarini 2007). This was evident with 4 and 20 μ g/ml doses of HHA, which were ineffective against HHA-500CS in CEM cell cultures (Balzarini 2007). This was reversed by DMJ (at both 20 and 50 μ M) which provided 100% protection from infection at the higher concentration of HHA (Balzarini 2007). Up to 80% protection was observed (with both doses of DMJ) at the HHA concentration of 4 μ g/ml (Balzarini 2007). Surprisingly, the synergistic effect of DMJ (at both doses) on the

HHA-500SN mutant only became operable at the 100 μ g/ml level screen of HHA (Balzarini 2007). DMJ alone was ineffective against wild-type HIV-1(III_B) (EC_{50} > 500 μ g/ml) and both mutant strains (EC_{50} s 155 and 103 μ g/ml, respectively), whilst the corresponding values for HHA were 0.31, 125 and 127 μ g/ml, respectively (Balzarini 2007). Synergistic effects for HHA against HIV-1(BaL) were probed in combination with the non-nucleoside reverse transcriptase inhibitor KRV2110 and the fusion inhibitor enfuvirtide (Jenabian et al. 2009). All three compounds individually inhibited viral growth in peripheral blood lymphocytes (PBLs), with IC_{50} values of 2.51, 0.53 and 1.59 μ g/ml, respectively (Jenabian et al. 2009). The effect of HHA (up to 12.5% of its IC_{50} dose) in combination with KRV2110 or enfuvirtide was shown to be synergistic as gathered from combination index (CI) values that were < 1 (Jenabian et al. 2009). Furthermore, the viabilities of host HeLa or HEC-1A (human endometrial adenocarcinoma) cells remained high at all dosage levels of testing (Jenabian et al. 2009).

HIV-1(III_B) strains produced from repeated exposure to the *Urtica dioica* agglutinin (UDA) in CEM cells (as noted above) were also tested for susceptibility to HHA (Balzarini et al. 2005b). The wild-type strain proved to be more sensitive to HHA (EC_{50} 0.006 μ M) than the seven mutants (mean EC_{50} 0.47 μ M), which were identified via mutations to amino acid residues at gp120 of the viral glycoprotein (Balzarini et al. 2005b). DS-5000 was markedly less active (EC_{50} 0.08 μ M, mean EC_{50} 0.15 μ M), but in which case the response between wild-type and mutant strains was not as pronounced (Balzarini et al. 2005b). Mutant strains of HIV-1(III_B) produced in response to CV-N displayed phenotypic resistance to CV-N that correlated with the number of glycan deletions in gp120 as described above (Balzarini et al. 2006). This was especially true for the mutants CV-N2a,113–114 and CV-N2b,113–115 (six mutations each) and CV-N2,108–4 (three mutations), which were much more resistant to CV-N than wild-type HIV-1(III_B) (Balzarini et al. 2006). HHA by comparison exhibited lower activity against the first two mutants (mean EC_{50} 0.055 μ M) as well as the third (EC_{50} 0.13 μ M), relative to CV-N (mean EC_{50} 0.040 μ M, EC_{50} 0.016 μ M) (Balzarini et al. 2006). Wild-type HIV-1(III_B) was most susceptible to HHA (EC_{50} 0.004 μ M), much more so than AMD-3100 (EC_{50} 0.05 μ M) or DS-5000 (EC_{50} 0.33 μ M) (Balzarini et al. 2006). The difference in the responses of CV-N and HHA could be rationalized in terms of a higher genetic barrier for the former (Balzarini et al. 2006).

The mutant HIV-1 NL4.3 (CADARes40) was produced from HIV-1(NL4.3) in MT-4 cells with increasing concentrations of CADA, an HIV entry inhibitor that restricts CD4 expression (Vermeire et al. 2009). Whilst wild-type HIV-1(NL4.3) displayed sensitivity to CADA, the mutant did not (EC_{50} s 1.2 and > 50 μ g/ml, respectively) (Vermeire et al.

2009). HHA by contrast was effective against both strains (EC_{50} s 0.11 and 0.15 $\mu\text{g/ml}$, respectively), where enfuvirtide (EC_{50} s 0.062 and 0.056 $\mu\text{g/ml}$, respectively) and indinavir (EC_{50} s 0.013 and 0.024 $\mu\text{g/ml}$, respectively) were used as reference standards (Vermeire et al. 2009). This suggested that Amaryllidaceae lectins such as HHA may be used in HIV/AIDS therapeutics against mutants that have become insensitive to conventional drugs (Vermeire et al. 2009).

Multiple passages of HIV-1(NL4.3) through culture with increasing concentrations of HHA or the HIV-1 antibody 2G12 selected for strains (HHA_{res} and $2G12_{res}$) that developed resistance to either (Mathys and Balzarini 2014). HHA was over 50-fold more potent against the wild-type NL4.3 strain (EC_{50} 0.010 μM) (in C8166 cell cultures) than it was towards the HHA_{res} mutant (EC_{50} 0.55 μM), whilst also demonstrating good activity against the mutant $2G12_{res}$ (EC_{50} 0.014 μM) (Mathys and Balzarini 2014). 2G12, whilst potentially active on wild-type HIV-1 (NL4.3) (EC_{50} 0.0051 μM), demonstrated diminished capacities against both mutants (EC_{50} 0.35 μM in each case) (Mathys and Balzarini 2014). In the case where the virus was passaged simultaneously with HHA and 2G12, the HHA/2G12 mutant was more susceptible to HHA (EC_{50} 0.17 μM) than to 2G12 (EC_{50} 0.35 μM) (Mathys and Balzarini 2014). Repeated exposure of HIV-1 cell cultures to HHA selected for mutants with deletions of several *N*-linked glycans on the gp120 envelope, mostly high-mannose-type glycans (such as Asn228, Asn232, Asn287 and Asn337) (Balzarini et al. 2004b; Wu and Bao 2013). The complex-type *N*-glycans deleted were also related to asparagine, notably positioned at Asn274, Asn299 and Asn395 (Balzarini et al. 2004b; Wu and Bao 2013). The neutralizing human antibody 2G12 is directed specifically towards HIV-1 gp120 and has specificity for clustered high-mannose-type glycans, particularly those with terminal $\alpha(1,2)$ -mannose residues (Scanlan et al. 2002). The high-mannose-type *N*-glycans on Asn293, Asn330, Asn337 and Asn390 are known to be instrumental both in the binding to HIV-1 gp120 and the neutralizing activity of 2G12 (Scanlan et al. 2002).

As noted above, I-IT and I-SPT are significant pathways in the transfer of HIV-1 to CD4+ lymphocytes, occurring via endocytic uptake and viral fusion (Turville et al. 2005). The sensitivities of these pathways to HHA were probed in monocyte-derived DCs, where potent inhibition of I-IT in HIV (strain Bal) was observed for the lectin (EC_{50} 0.007 $\mu\text{g/ml}$) (Turville et al. 2005). By contrast, blockage of I-SPT was harder to achieve as shown by an EC_{50} of 2.60 $\mu\text{g/ml}$ for inhibition of the pathway by HHA in HIV (strain Bal) (Turville et al. 2005).

Hippeastrum hybrid agglutinin was also the most active of the three lectins examined against SIV (mac251) (Retroviridae) (EC_{50} 0.3 $\mu\text{g/ml}$), four times more so than DS-10000 (EC_{50} 1.2 $\mu\text{g/ml}$), but like GNA and NPA it proved to be less

effective against SRV and MSV (EC_{50} s 40 and 32 $\mu\text{g/ml}$, respectively) (Balzarini et al. 1991). HHA was also active against FIV (Petaluma) (EC_{50} 0.16 $\mu\text{g/ml}$), more so than tenofovir (EC_{50} 2.3 $\mu\text{g/ml}$) (Balzarini et al. 2004a). HHA inhibited syncytium formation between SIV(mac251)-infected Raji/DC-SIGN and uninfected C8166 lymphoblasts (IC_{50} 0.032 μM), compared to pradimicin-A which produced the effect at a much higher concentration (IC_{50} 19 μM) (Francois et al. 2008). The mutant PRM-A-1, generated from SIV(mac251) in MT-4 cultures by increasing pradimicin-A exposure and characterized by mutations on the gp120 envelope, also succumbed to HHA (EC_{50} 0.034 μM) (Francois et al. 2008). This represented a tenfold betterment of the response seen for DS-5000 (EC_{50} 0.35 μM) (Francois et al. 2008). However, the wild-type strain was much more sensitive to HHA (EC_{50} 0.002 μM) than the mutant, with DS-5000 not reflecting a significant difference in its response to either (Francois et al. 2008). HHA (at 1 and 10 $\mu\text{g/ml}$) delayed the peak for SIV(mac239) replication by 8 days in CEMx174 cells (Stansell and Desrosiers 2010). The passage of SIV(mac239) through culture under increasing HHA concentrations (0.1 to 200 $\mu\text{g/ml}$) yielded a resistant population, wherein three sites for *N*-linked carbohydrates on the envelope protein were deleted (Stansell and Desrosiers 2010). As noted with GNA above, two of the sites were in the gp120 subunit whilst the remaining one was located on the envelope gp41 transmembrane protein (Stansell and Desrosiers 2010). Furthermore, gp120 from the resistant variant exhibited a far lower binding capacity for HHA than the mac239 gp120 (Stansell and Desrosiers 2010).

Infection of PBMCs by human T lymphotropic virus (HTLV) (Retroviridae) type 1 can occur by co-cultivation of the cells with irradiated HTLV-1 transformed MT-2 leukocytes (Balestrieri et al. 2008). This model can serve as a rigid mimic of HTLV-1 transmission via cell-to-cell contact (Balestrieri et al. 2008). The absence of virus-specific mRNA and the viral protein Tax in the PBMC acceptor cells following exposure to HHA at non-toxic concentrations ($> 2 \mu\text{M}$) suggested cell-to-cell transmission of HTLV-1 can be inhibited by the lectin (Balestrieri et al. 2008). The non-peptidic $\alpha(1,2)$ -mannose-specific antibiotic pradimicin-A by comparison was tolerated at much higher concentrations ($CC_{50} > 120 \mu\text{M}$) (Balestrieri et al. 2008). Furthermore, the effect of HHA was sustainable when HTLV-1-infected C5MJ lymphocytes replaced the MT-2 leukocytes as a virus donor cell line (Balestrieri et al. 2008).

Hippeastrum hybrid agglutinin has also been evaluated for effects against the Herpesviridae, notably CMV infection of HEL cells (Balzarini et al. 1991). With respective EC_{50} values of 0.87 and 1.5 $\mu\text{g/ml}$ against the AD169 and Davis strains, it was slightly less effective than DHPG (mean EC_{50} 0.79) (Balzarini et al. 1991). The response was boosted by

the fact that individually, both entities were relatively harmless to HEL host cells (MTCs > 100 µg/ml) (Balzarini et al. 1991). Herpes simplex virus (Herpesviridae) has also been exposed to HHA, whose HSV-1 and HSV-2 strains were moderately resistant to the lectin (EC_{50} s > 1 µM) (Bertaux et al. 2007). The mean EC_{50} for DS-5000 against both strains was 0.8 µM (Bertaux et al. 2007). Furthermore, the effect on host HEL cells was milder with DS-5000 (CC_{50} > 100 µM) than with HHA (CC_{50} > 2 µM) (Bertaux et al. 2007). Feline herpes virus (FHV) succumbed to HHA (EC_{50} 0.22 µM), without severe effects being manifested on Crandell-Rees feline kidney (CRFK) host cells (CC_{50} > 50 µg/ml) (Gordts et al. 2015).

Hippeastrum hybrid agglutinin exhibited potent inhibitory effects against HCV (Flaviviridae) proliferation in Huh-7 cells (EC_{50} 0.003 µM), more so than DS-5000 (EC_{50} > 50 µM) and with minimal effect on the host (CC_{50} > 10 µM) (Bertaux et al. 2007). However, its antiviral effect fell off sharply (to EC_{50} > 50 µM) in the presence of mannan (2.5 mg/ml) (Bertaux et al. 2007). In addition, HHA effectively blocked entry (EC_{50} 0.0006 µM) of HCV pseudoparticles (HCVpp) into Huh-7 cells (Bertaux et al. 2007). A further pathogen from the Flaviviridae to be exposed to HHA was dengue virus (Alen et al. 2009). HHA potently inhibited infection of both Raji/DC-SIGN and IL-4-treated monocytic cells (EC_{50} s 0.11 and 0.0044 µM, respectively), a response that ensued without detriment to the host cells (CC_{50} s > 5000 µM in each case) (Alen et al. 2009). Raji/0 human B cells by comparison were not affected by the virus, highlighting the significance of DC-SIGN as a binding receptor for dengue virus (Alen et al. 2009). Exposure of human monocyte cells to the cytokine IL-4 leads to the expression of DC-SIGN, hence their susceptibility to infection (Alen et al. 2009). Furthermore, naturally susceptible Vero cells could not be protected by HHA (EC_{50} > 5000 µM), suggesting that viral entry was dependent on cell type (Alen et al. 2009). HHA was effective against four serotypes of dengue virus (DENV-1, DENV-2, DENV-3 and DENV-4) as shown via its potent inhibition of viral replication in MDDCs (EC_{50} s 0.017, 0.0046, 0.014, 0.011 µM, respectively) (Alen et al. 2011). Substitution of MDDCs for Raji/DC-SIGN cells led to notable losses in activity across all four pathogens, which nevertheless remained at the micromolar level (EC_{50} s 0.054, 0.034, 0.029 and 0.092 µM, respectively) (Alen et al. 2011). DENV-2 replication in MDDCs was also inhibited by pradimicin-S, but at comparatively higher doses (EC_{50} 11.0 µM) (Alen et al. 2011). Furthermore, it was ineffective against DENV-2 infection of Raji/DC-SIGN cells (EC_{50} > 55 µM) (Alen et al. 2011). Intervention by HHA was at an early stage of DENV-2 infection of Raji/DC-SIGN cells as seen by its ability to block viral attachment by binding to the viral envelope (Alen et al. 2011). Furthermore, HHA displayed weak activity when it

was administered after viral attachment (Alen et al. 2011). The levels of CD80, CD86 and DC-SIGN in DENV-2-infected MDDCs following exposure to HHA (0.4 µM) were almost identical to those detected in uninfected cell cultures (Alen et al. 2011). This suggested that HHA also prevented cellular activation and differentiation in MDDCs that were induced following DENV-2 infection (Alen et al. 2011).

A dengue virus strain (DENV HHA^{res}) resistant to HHA was generated in the C6/36 mosquito cell line (Alen et al. 2012). Sequencing of the structural proteins of HHA^{res} relative to the wild-type strain indicated the presence of two mutations (N67D and T155I), which emerged from the deletion of both *N*-glycosylation sites on the E-glycoprotein of the viral envelope (Alen et al. 2012). Furthermore, the mutant could replicate in a variety of mammalian (Vero, Huh-7, BHK) and mosquito (C6/36) cells that were lacking DC-SIGN expression (Alen et al. 2012). By contrast, DC-SIGN+ (Raji/DC-SIGN) cells, cells that expressed the DC-SIGN-related liver-specific receptor L-SIGN (Raji/L-SIGN) or MDDCs were not susceptible to DENV HHA^{res} (Alen et al. 2012). *N*-glycans in the E-glycoprotein thus play a crucial role in the infection of dendritic cells, which constitute primary target cells for dengue virus during viral pathogenesis (Alen et al. 2012).

Screening of the family Rhabdoviridae showed that HHA was active against VSV (EC_{50} 0.4 µM) relative to DS-5000 (EC_{50} 0.8 µM), in the process also proving to be ineffective on HeLa host cells (CC_{50} > 2 µM) (Bertaux et al. 2007). HHA and DS-5000 exhibited the same inhibitory effects on RSV (Pneumoviridae) as they did on VSV above (EC_{50} s 0.4 and 0.8 µM, respectively) (Bertaux et al. 2007). However, the former (CC_{50} > 2 µM) was noted for a much stronger response to host HeLa cells than the latter (CC_{50} > 100 µM) (Bertaux et al. 2007). The parainfluenza-3 virus (Paramyxoviridae) succumbed more readily to HHA (EC_{50} 2 µM) than to DS-5000 (EC_{50} > 100 µM), with a more pronounced effect being recorded for the former (CC_{50} > 2 µM) than the latter (CC_{50} > 100 µM) against host Vero cells (Bertaux et al. 2007).

Hippeastrum hybrid agglutinin was seen to be the most effective of the four Amaryllidaceae lectins probed for inhibition of SARS-CoV (Frankfurt 1) and FIPV (79–1146) (Coronaviridae) infection of monkey kidney Vero and Crandell-Rees feline kidney cells, respectively (Keyaerts et al. 2007). EC_{50} values of 3.2 and 2.6 µg/ml were obtained for HHA against the viruses (Table S2), whilst proving to be far less detrimental to the respective host cells (CC_{50} s > 100 µg/ml) (Keyaerts et al. 2007). Twelve *N*-glycosylation sites have been identified on the SARS-CoV spike protein (Krokhin et al. 2003). Two of the four sugars attached to four of the sites were high-mannose-type glycans, the other two being complex glycan structures (Krokhin et al. 2003). The presence of such high-mannose-type glycans has been used to

explain the anti-SARS-CoV activity of mannose-specific HHA (Keyaerts et al. 2007). Furthermore, pre-exposure of either SARS-CoV or Vero cells to HHA offered no increased protection from virus-induced cytopathicity (Keyaerts et al. 2007). It was suggested that this could be due to a lower binding affinity of HHA to the coronavirus envelope glycans and/or a faster detachment of the lectin from the envelope glycans (Keyaerts et al. 2007). HHA exhibited potent activity against the TN406HP strain of FIPV (EC_{50} 0.007 μ M) relative to pradimicin-A (EC_{50} 2.5 μ M), the effect of which was mild on FCWF host cells (CC_{50} > 2 μ M) (Van der Meer et al. 2007a). A significant drop in activity was noted following exposure of HHA to FIPV strain 79–1146 (EC_{50} 0.10 μ M), which was more pronounced for pradimicin-A (EC_{50} > 120 μ M) (Van der Meer et al. 2007a). HHA (at 50 μ g/ml) also reduced syncytium formation in FCWF cells that were infected by the 79–1146 strain (Van der Meer et al. 2007b).

Syncytium formation in LR7 mouse fibroblasts infected by mouse hepatitis virus (MHV) (strain A59) was also inhibited by HHA at 50 μ g/ml (Van der Meer et al. 2007b). This suggested that the effect of HHA may occur via binding to the S-protein, whose expression on the cell surface ushers in cell fusion and syncytium formation (Van der Meer et al. 2007b). HHA has also been examined for effects on the coronavirus M-protein, which by contrast plays no role in these processes (Van der Meer et al. 2007b). The MHV mutant strains Alb248, Alb138 and Alb244 were utilized since they express M-proteins with no sugar moieties, O-linked sugars and N-linked sugars, respectively (Van der Meer et al. 2007b). The Alb248 and Alb138 strains proved to be most susceptible to HHA (EC_{50} s 1.2 and 1.8 μ g/ml, respectively), with the EC_{50} value shown to be 2.9 μ g/ml for the remaining mutant (Van der Meer et al. 2007b). EC_{50} values of 2, 5.7 and 4.1 μ g/ml, respectively, were determined in the comparison against pradimicin-A (Van der Meer et al. 2007b). The results for HHA suggested that it may also be using the M-protein in its targeted response to the coronavirus MHV (Van der Meer et al. 2007b).

FCWF cells were also host to FCoV (strain 79–1683) against which HHA displayed submicromolar level activity (EC_{50} 0.11 μ M) compared to pradimicin-A (EC_{50} > 120 μ M) (Van der Meer et al. 2007a). IBV (strain Beaudette) infection of Vero cells was strongly inhibited by HHA (EC_{50} 0.0002 μ M), much more so than by pradimicin-A (EC_{50} 2.9 μ M) (Van der Meer et al. 2007a). By contrast, the cytotoxic effect on the host by the former (CC_{50} > 2 μ M) was greater than the latter (CC_{50} 120 μ M) (Van der Meer et al. 2007a). TGEV (strain Purdue) proliferation in swine testicular cells was markedly inhibited by HHA (EC_{50} 0.004 μ M) relative to pradimicin-A (EC_{50} 4.7 μ M), with their cytotoxic effects shown to be the same as those demonstrated on Vero cells above (Van der Meer et al. 2007a).

Studies on the family Tobaniviridae involved the Berne virus in equine dermis cells, against which HHA exhibited good activity (EC_{50} 0.07 μ M) in comparison to pradimicin-A (EC_{50} 31 μ M) (Van der Meer et al. 2007a). The difference in activity for each compound on the host was strikingly large as seen via CC_{50} values of > 2 and > 120 μ M, respectively (Van der Meer et al. 2007a). The family Arteriviridae was also probed for infectivity of equine dermis cells (Van der Meer et al. 2007a). In particular, EAV proved to be more susceptible to HHA (EC_{50} 0.13 μ M) than to pradimicin-A (EC_{50} > 120 μ M) (Van der Meer et al. 2007a). HHA has also been examined for effects on influenza viruses (Orthomyxoviridae), whereby its inhibition of H3N2 proliferation in MDCK cells was far more pronounced than for H1N1 (EC_{50} s 0.002 and 0.54 μ M, respectively) (Gordts et al. 2015). Influenza B virus, similar to the influenza A viruses H1N1 and H3N2, also succumbed to HHA exposure in the self same host cells (EC_{50} 0.006 μ M) (Gordts et al. 2015). Coxsackie B4 virus (Picornaviridae) and reovirus-1 (Reoviridae) proliferation were not significantly affected by HHA (EC_{50} s > 2 μ M), neither were host HeLa and Vero cells (MCCs 100 μ g/ml) (Gordts et al. 2015).

***Leucojum aestivum* Agglutinin**

The *Leucojum aestivum* agglutinin (LAA) bore all of the features seen for NPA, indicating it to also be a dimer of two 13-kDa subunits (Van Damme et al. 1988). However, ion exchange chromatography indicated it to be a complex mixture of isolectins, all of which nonetheless gave a single polypeptide band of 13 kDa after SDS-PAGE analysis (Van Damme et al. 1988). The first examination of LAA for antiviral effects was in regard to HIV-1(III_B) (Retroviridae) infection of C8166 cells (Animashaun et al. 1993). It inhibited syncytium formation (at 100 μ g/ml) in the host cells relative to AZT, with the structures being apparent at the two lower tested concentrations (1 and 10 μ g/ml, respectively) (Animashaun et al. 1993). The virus was also dose-dependently depleted by LAA, with the viral progenies relative to control shown to be 100, 50 and 0% at 1, 10 and 100 μ g/ml, respectively (Animashaun et al. 1993). Whilst uninfected host cells were fully viable at all three levels, infected cells reflected viabilities of 19, 62 and 100% at the three dosages, respectively (Animashaun et al. 1993).

***Lycoris radiata* Agglutinin**

Purified *Lycoris radiata* agglutinin (LRA) showed a single protein band via SDS-PAGE analysis, with a subunit molecular weight of 12 kDa (Chang et al. 2005). The relative molecular weight of LRA was determined to be 45 kDa, suggesting it to exist as a tetramer of four individual subunits (Chang et al. 2005). It was noted for its rabbit

erythrocyte and *Escherichia coli* agglutinating activities, with 0.95 and 1.90 µg/ml being the minimum concentrations required for the effects, respectively (Chang et al. 2005). The agglutinating effect was readily reversed by mannan and thyroglobulin (Chang et al. 2005). LRA was then examined for effects against HSV-2 (Herpesviridae), where it dose-dependently inhibited proliferation of the virus (IC₅₀ 7.5 µg/ml) without cytotoxicity towards Vero host cells (IC₅₀ > 500 µg/ml) (Chang et al. 2005). It displayed weak activity against SARS-CoV (Frankfurt 1) (Coronaviridae) (EC₅₀ 48 µg/ml), also proving to be non-toxic to the host Vero cells (CC₅₀ > 100 µg/ml) (Keyaerts et al. 2007). LRA was inactive against FIPV(79–1146) (Coronaviridae) as well as Crandell-Rees feline kidney cells that were exploited as hosts (Keyaerts et al. 2007).

***Crinum latifolium* Agglutinin**

Crinum latifolium agglutinin (CLA) was identified as a mannose-binding Amaryllidaceae lectin, with a specificity for methyl- α -D-pyranoside (Kaur et al. 2008). It adopted a homodimeric form to its structure (M_r 24 kDa) that did not incorporate disulphide linkages, with each of its subunits reflecting a mass of 12 kDa (Kaur et al. 2008). The antiviral component of its biological examination involved a screen against the vaccinia virus (strain vGK5) (Poxviridae) that was cultured on BSC-1 monkey kidney epithelial cells (Kaur et al. 2008). Viral replication was dose-dependently inhibited by CLA, with 73% inhibition observed at the highest tested concentration (34.4 µg/ml) (Kaur et al. 2008). Furthermore, no deleterious effects were manifested on the host cells at any of the tested dosages (34.4, 17.2 or 8.6 µg/ml, respectively) (Kaur et al. 2008).

Perspectives and Future Directions

Since antiviral lectins have thus far been described chiefly out of the genera *Galanthus*, *Narcissus* and *Hippeastrum* and given there are about eighty genera and around one thousand species in the Amaryllidaceae, future efforts will no doubt be targeted towards the remaining members of the family. Since only thirteen viral families (out of over two hundred) have been represented in screening measures against Amaryllidaceae lectins, the space is vast for exploration against the outstanding family members. Whilst there were useful insights afforded on the ways in which the lectins manifested their action, most of these were based on models of HIV infectivity. Their modes of action against pathogens from other families thus also remain a vast area for future investigation. Given their efficacies and selectivities, there was

surprisingly only two attempts to establish the performance of these lectins via *in vivo* models of study. This will surely be addressed in endeavours going forward.

Conclusions

This survey showcases the plant family Amaryllidaceae as a viable source of antiviral lectin principles. These have demonstrated potent inhibitory effects against a significant array of viruses, including both animal and human pathogens. Such manifestations were without detriment to the majority of cells that were used as hosts. Although good effects were observed across the viral families examined, the most promising were those against HIV of the Retroviridae. It is thus in HIV/AIDS that the potential of these lectins as antiviral drugs may be truly realized.

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